Brassinosteroids and their Biological Activities

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Jana Oklešťková, Lucie Rárová, and Miroslav Strnad

Contents

Abstract

Brassinosteroids are endogenous plant polyhydroxysteroids that are essential for normal plant growth and development. They induce cell elongation and division, increase DNA and RNA polymerase activity, interact synergistically with

J. Oklešťková (✉) • L. Rárová
Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic
e-mail: jana.oklestkova@upol.cz; lucie.rarova@upol.cz

M. Strnad
Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic

Department of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, Czech Republic
e-mail: miroslav.strnad@upol.cz

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auxins, stimulate ethylene production, and increase tolerance to temperature, water, and salinity stress. They are observed in many plant species and are present in nearly every part of the plant, although the highest concentrations occur in the reproductive organs. In mammalian cells, brassinosteroids inhibit cell growth and affect cell cycle progression; as such, they have potential for development as anticancer agents. Moreover, brassinosteroids and their synthetic derivatives possess antiangiogenic properties that could be effective in the treatment of any carcinoma. As such, they are an interesting source of lead compounds for developing novel natural product-derived anticancer drugs, and their properties have attracted the attention of many specialists in the fields of chemistry, biochemistry, pharmacology, plant physiology, and agriculture.

Keywords

Anticancer activity • apoptosis • bioassay • biosynthesis • brassinosteroids • HPLC/MS • signaling pathway • stress protection

Abbreviations

24-epiBL	24-epibrassinolide
28-homoBL	28-homobrassinolide
28-homoCS	28-homocastasterone
BL	Brassinolide
BRs	Brassinosteroids
CS	Castasterone
FW	Fresh weight
GA_3	Gibberellic acid

1 Introduction

The role of steroids as mammalian hormones has been known since 1930, and steroidal hormones have also been found in insects and fungi. Plants can biosynthesize a large variety of steroids, but it was not known until 1979 that steroids with plant growth-promoting activity were discovered. In that year, Grove et al. [1] reported the discovery of a new steroidal lactone called brassinolide from the pollen of *Brassica napus* L. To date, more than 70 structurally and functionally related steroids have been isolated from plant materials [2]. These compounds have been identified as members of a new group of plant hormones – the brassinosteroids (BRs).

BRs have been detected in all plant organs, including pollen, anthers, seeds, leaves, stems, roots, flowers, and grains. They have also been found in other interesting tissues such as insect and crown galls, notably the galls of *Castanea crenata, Distylium racemosum*, and *Catharanthus roseus*. Pollen and immature seeds in particular tend to be especially rich source of BRs, whereas their concentrations in vegetative tissue are very low compared to other plant hormones. Pollen

and immature seeds contain about 1–100 ng.g^{-1} FW of BRs, while shoots and leaves usually have lower amounts (0.01–0.1 ng.g^{-1} FW) [3].

BRs control many developmental and physiological processes in plants, including regulation of gene expression, cell division and expansion, germination, vegetative and reproductive development, vascular differentiation, root growth, programmed cell death, and homeostasis [4, 5]. In addition to their growth-regulating activities, BRs have been shown to have dynamic roles in protecting plants against biotic and abiotic stresses [6, 7]. Treatment with exogenous BRs raises the inner potential of treated plants, which promotes survival under stressful conditions and also reduces biotic stress caused by pathogens [5, 8].

The physiological concentrations of BRs in plants are extremely low (ng.Kg^{-1}Fw), and it can be very difficult to analyze their abundance in plant tissues. A wide range of methods are currently employed for the determination and quantification of brassinosteroids in plants, including bioassays, diverse chromatographic procedures, radioimmunoassays [9], and enzyme-linked immunosorbent assays [10–13]. The most widely used bioassays are the second bean internode bioassay and rice–lamina inclination test. These bioassays have been used in the isolation of brassinolide from rape pollen [1] and castasterone from chestnut insect galls [14]. Gas chromatography–mass spectrometry (GC–MS) analysis is the current standard technique for instrumental analysis of BRs [15–17].

Some medically oriented applications of BRs have been reported [8, 18, 19]. Wachsman et al. [8, 20] observed that some natural BRs (28-homoCS, 28-homoBL) and their synthetic analogs exhibit antiviral activity in vitro against several RNA and DNA viruses, including herpes simplex virus type 1 (HSV-1), arenaviruses, and measles virus (MV). Moreover, natural and synthetic brassinosteroids have been reported to cause growth inhibition, cell cycle arrest, and initiation of apoptosis in many different cancer cell lines. It has also been demonstrated that BRs can inhibit the proliferation and migration of human endothelial cells, and there is evidence that BR treatment triggers the initiation of cell death by apoptosis. Brassinosteroid analogs have proven to be more effective than natural BRs in this context. Importantly, BRs inhibit cell migration and tube formation, suggesting they may possess antiangiogenic activity [21–23].

2 Chemical Structures of the Brassinosteroids

All known brassinosteroids have a 5α-cholestane, 5α-ergostane, or 5α-sitostane steroidal skeleton with mono- to trioxygenation on ring A and 22α-, 23α-dihydroxylation in the side chain. Ring B may be fully saturated or may contain a ketone or lactone at carbon 6 (Fig. 128.1).

All natural BRs consist exclusively of carbon, hydrogen, and oxygen and have:

1. A normal or B-homo cholestane, ergostane, or sitostane steroidal skeleton
2. An oxygen-containing functional group at carbon 3 (α- or β-hydroxyl, ester, ether, or ketone)
3. An all-*trans* fusion between rings A/B, B/C, and C/D

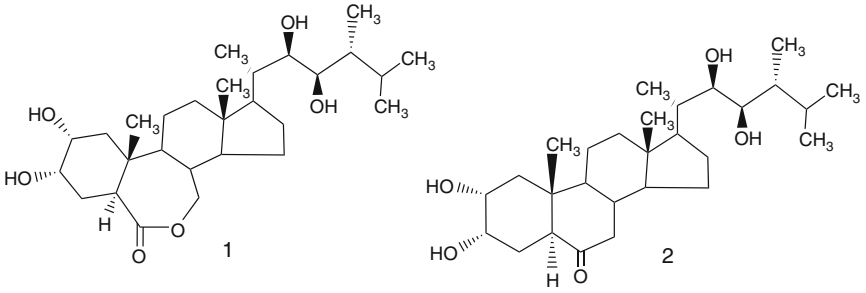


Fig. 128.1 Structures of brassinolide (1) and castasterone (2)

4. A 20R (or 20 β) configuration
5. α –*cis* (R,R) vicinal hydroxyls at carbons 22 and 23

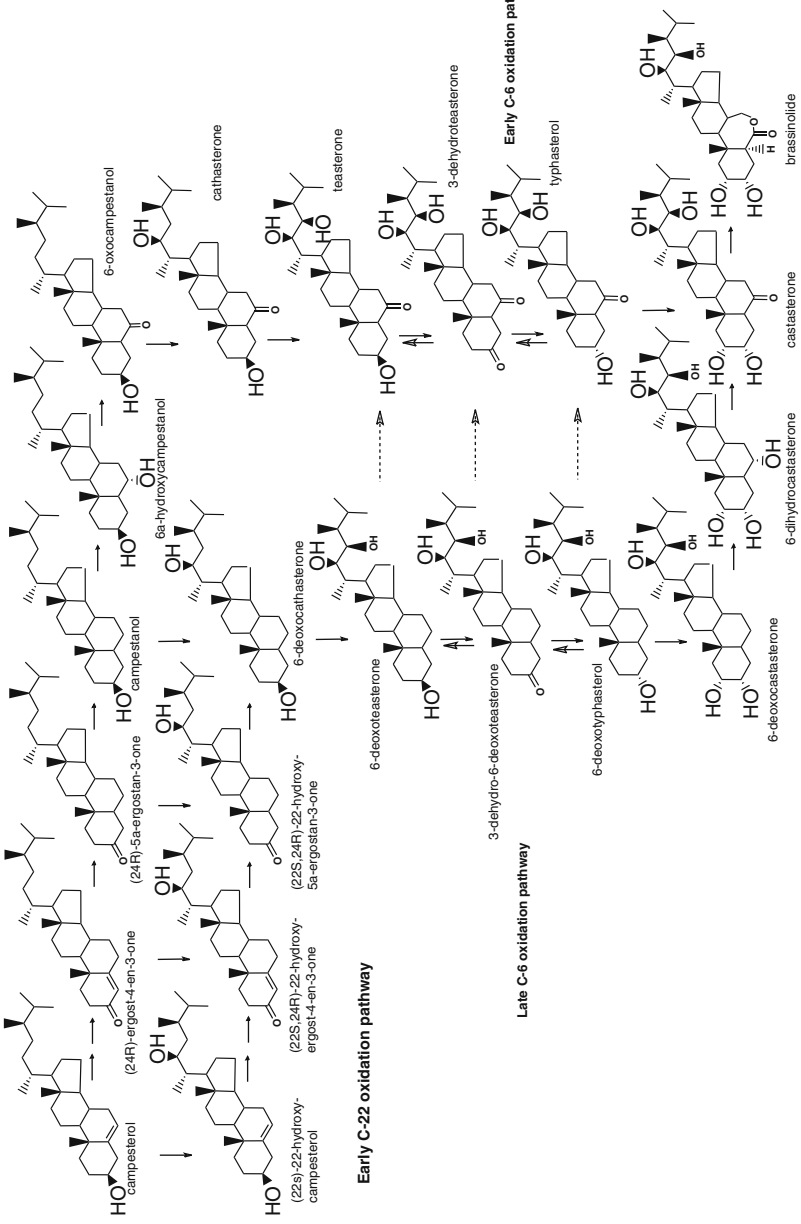
All of these compounds have an oxygen-containing functional group at carbon 3, none exhibit A/B *cis* ring fusion or have a carbon–carbon double bond between rings A and B, and all have at least 27 carbon atoms [24, 25].

3 Biosynthetic Pathway

BRs belong to the class of molecules known as triterpenoids. Because BRs are a group of modified sterols, the BR biosynthetic pathway can be divided into two major parts: the sterol-specific pathway (which converts squalene to campesterol) and the BR-specific pathway (which converts campesterol to the BR). In the sterol-specific pathway, mevalonic acid (which is the starting material in terpenoid biosynthesis) is condensed and cyclized to produce 2,3-oxidosqualene. This precursor is further modified to form the major plant sterols such as sitosterol and campesterol. To become bioactive BRs, sterols must be processed by the BR-specific pathway.

The biosynthetic pathways leading to brassinolide were initially elucidated using cultured *Catharanthus roseus* cells. Extensive metabolic studies suggested the operation of a series of parallel branched biosynthetic pathways that were named the early and late C-6 oxidation pathways (Scheme 128.1) [26–28]. Recent studies suggest that there is cross-talk between these parallel pathways, implying that they are not totally autonomous. The existence of an early C-22 oxidation branch in the BR biosynthetic pathway has also been demonstrated. It thus appears that BR biosynthetic pathways are highly networked [29–31].

As shown in Scheme 128.1, campesterol is first converted to campestanol, which is then converted to castasterone via either early or late C-6 oxidation. Finally, castasterone is converted to brassinolide. However, some of the steps involved have only recently been clarified. One such step, the conversion of 6-oxocampestanol to cathasterone, was demonstrated in cultured *Catharanthus roseus* cells [32], fully substantiating the pathway from campestanol to



Scheme 128.1 Pathways of brassinosteroid biosynthesis (Modified by Fujioka S, Yokota T [105])

brassinolide via early C-6 oxidation. The campestanol → 6-deoxocathasterone → 6-deoxoteasterone sequence in the late C-6 oxidation pathway has also been established in *Arabidopsis* [31, 33], completing the elucidation of the late C-6 oxidation pathway. Ohnishi and coworkers proposed a novel shortcut in BR biosynthesis in *Arabidopsis*, which allows the direct conversion of early 22-hydroxylated intermediates to 3-dehydro-6-deoxoteasterone and 6-deoxotyhpasterol via C-23 hydroxylation [34]. In tomato and tobacco, the late C-6 oxidation pathway appears to be the primary route used because the only endogenous BRs in these species are produced via the late C-6 oxidation pathway [35, 36].

4 Brassinosteroid Signal Transduction

The perception of signals at the cell surface and the transduction of these signals to the cell's interior are essential in all life forms. In plants, this process is mediated by membrane-integral receptor kinases [37]. Brassinosteroid signaling (Fig. 128.2) and the resulting genomic response is initiated by the binding of a BR molecule to a receptor kinase, brassinosteroid-insensitive 1 (BRI1), which is localized in the plasma membrane. BRs bind to the extracellular leucine-rich repeat receptor kinase (LRR-RK) domain of BRI1. This triggers the phosphorylation of the intercellular serine–threonine kinase domain of BRI1, which in turn causes BRI1 to dissociate from the membrane-bound BRI1 kinase inhibitor 1 (BKI1) and to form a complex with a second receptor kinase, BRI1-associated receptor kinase 1 (BAK1). The active BRI1/BAK1 receptor kinase pair then propagates the signal downstream by inactivating a soluble kinase, brassinosteroid-insensitive 2 (BIN2), which is a negative regulator of BR signaling [39–42]. BKI1 is phosphorylated at a tyrosine residue in response to brassinosteroid perception; this mechanism is conserved in higher organisms and controls protein localization. The phosphorylated residue is part of a membrane-targeting motif and its modification releases BKI1 into the cytosol, allowing it to form an active signaling complex [43]. The structure of the *Arabidopsis thaliana* BRI1 ligand-binding domain has recently been published; it features a superhelix of 25 twisted leucine-rich repeats (LRRs) that is strikingly different to the assembly of LRRs in animal Toll-like receptors [37, 44]. A 70-amino-acid island domain between LRRs 21 and 22 folds back into the interior of the superhelix to create a surface pocket that contains the binding site for the plant hormone brassinolide. It seems that steroid binding to BRI1 generates a docking platform for a coreceptor that is required for receptor activation [37]. Irani et al. [45] used chemical and genetic approaches to interfere with the trafficking of the BRI1–BR complexes and examined their effect on BR signaling. They developed a bioactive, fluorescent BR analog (AFCS) and used it to visualize the endocytosis of BRI1–AFCS complexes in living *Arabidopsis* cells. These studies revealed that interference with clathrin- or ARF-GEF-mediated endocytosis of BRI1 enhanced BR signaling [45].

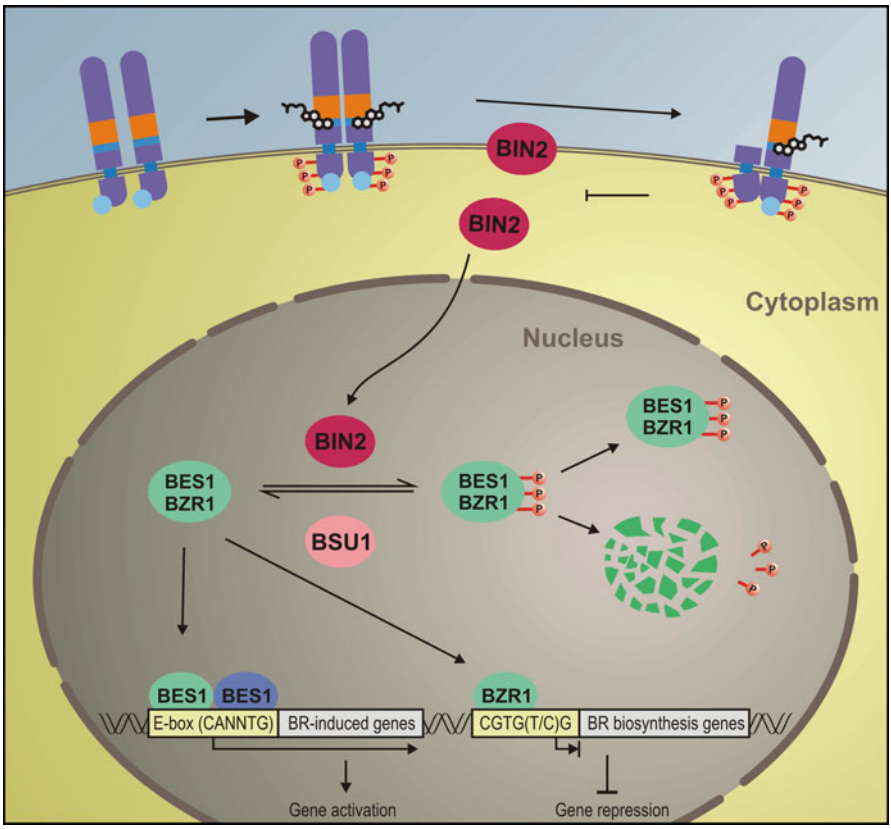


Fig. 128.2 Brassinosteroid signaling in the plant cell. BRs bind to the extracellular domain of brassinosteroid-insensitive 1 (BRI1), a leucine-rich repeat receptor kinase (LRR-RK) that is localized in the plasma membrane. This leads to phosphorylation of the intracellular serine–threonine kinase domain of BRI1, causing it to dissociate from the membrane-bound BRI1 kinase inhibitor 1 (BKI1) and to form a complex with a second receptor kinase, BRI1-associated receptor kinase 1 (BAK1). The active BRI1/BAK1 receptor kinase pair then propagates the signal downstream by inactivating a soluble kinase, brassinosteroid-insensitive 2 (BIN2), which is a negative regulator of BR signaling. BES1 (bri1-EMS-suppressor 1) and BZR1 (brassinazole-resistant 1) are phosphorylated by BIN2 and are closely related transcriptional activators of BR-induced genes. BSU1 (bri1 suppressor 1) counteracts the effects of BIN2 [38]

24-Epibrassinolide (24-epiBL) has also been shown to upregulate the transcription of an important positive cell cycle regulator gene, cyclin D3, which is involved in the activation of cell division by cytokinins in *Arabidopsis thaliana* L. [46]. No plant gene encoding an intracellular BR receptor has yet been identified in plants, although a chaperone heterocomplex that resembles an animal intracellular steroid receptor has been identified in wheat germ lysate [47]. Nevertheless, it is likely that there is an intracellular steroid hormone signaling pathway in plants that regulates gene transcription in a similar way to that found in animals [46].

5 Biochemical Analysis of Natural Brassinosteroids

5.1 Bioassays

The development of bioassays for isolating bioactive compounds from natural sources has played an important role in recent studies of natural BR phytochemistry. The development of highly sensitive and specific bioassays was essential for the isolation and purification of BRs from plant tissues because of the very low physiological concentrations of these hormones. The bean second internode assay was used to isolate BL from rape pollen [1], and the rice–lamina inclination test was used to isolate CS from chestnut insect galls [14]. Following the publication of these results, the rice–lamina inclination test has been widely used to isolate many BRs from various plant sources because of its simplicity, high sensitivity, and specificity for BRs [25].

5.1.1 The Bean Second Internode Bioassay

This test was first used for monitoring the biological activity of separated fractions during the isolation and purification of the "brassin-complex" [48–50] and later also for isolating pure BL [1]. Both gibberellins and BRs are active in this test, but the former cause only elongation, whereas BRs also cause swelling, increased curvature, and splitting of the internodes. Responses of this kind are considered indicative of "brassin activity" and are only observed for fractions containing BRs. The effect depends on the amount of BR applied. For example, swelling and increased curvature are only observed after treatment with at least 0.01 μg BL per plant. The sensitivity of the assay can be increased by omitting nitrate from and adding Ca^{2+} or Mn^{2+} to the solution in which the bean seedlings are grown [51].

5.1.2 The Rice-Lamina Inclination Bioassay

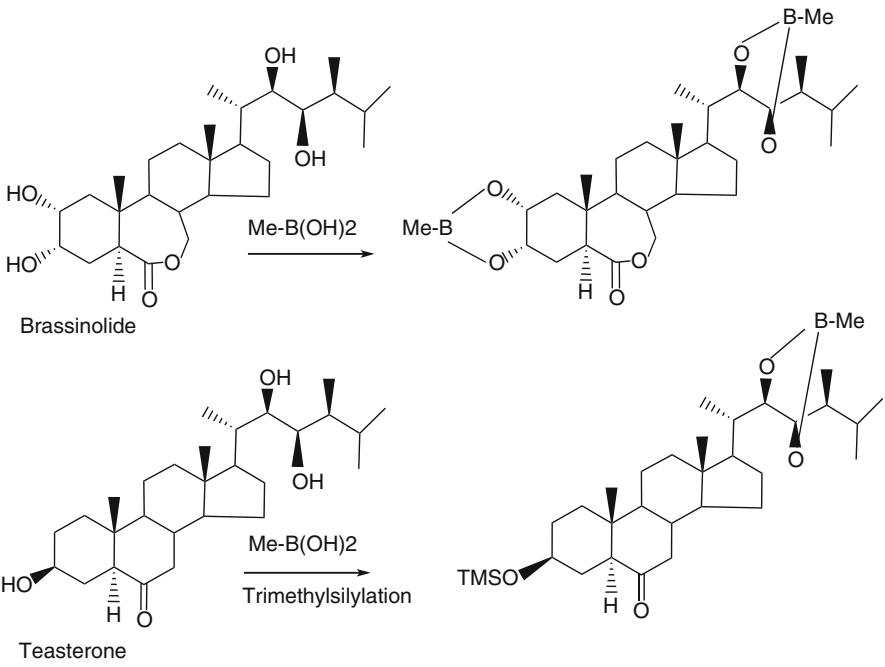
This bioassay is one of the most popular and useful tests for evaluating brassinosteroid activity. It was originally developed by Maeda [52] for gibberellins and indole-3-acetic acid (IAA). Two modifications were developed for use with brassinosteroids, one involving intact plants [53] and the other involving cuttings [54]. The assay is based on measuring the angles between the laminae and the sheaths after a period of incubation with the tested fraction; in the control segments, these angles are approximately 90°. In the presence of 0.0005 μg/ml BL, the angles between laminae and sheaths increased to around 140°; 0.005 μg/ml BL caused pronounced bending at the lamina joints to the abaxial side to the extent that some of the laminae came into contact with the sheaths. The activity of 28-homobrassinolide in this assay was comparable to that of BL, but the response induced by IAA was very modest even at 50 μg/ml [54]. Gibberellins also have only a modest effect on the inclination of the lamina in rice seedlings at a concentration of approximately 100 μg/ml. The sensitivity of the test depends on the rice cultivars used; out of 60 cultivars that were tested during its development, Arborio J-1 and Nihinbare were found to be the most reliable [55].

5.2 Gas Chromatography–Mass Spectrometry (GC–MS)

Brassinosteroids are highly polar and nonvolatile compounds. As such, it is generally necessary to convert them into more volatile derivatives prior to conducting gas-phase analyses. One such gas-phase analytical procedure that has proven useful when trying to identify BRs in a partially purified bioactive fraction is gas chromatography coupled to mass spectrometry in selected ion-monitoring mode (GC–MS-SIM). To facilitate their analysis by gas chromatography, BRs are typically converted into volatile bismethaneboronate (BMB) derivatives by treating them with methylboronic acid, which reacts specifically with vicinal diols (Scheme 128.2). This derivatization process makes it easy to separate the target compounds from contaminants that do not contain vicinal diols. The BMB derivatives are suitable for gas-phase analysis and also for the analysis of fragment ions in electron impact (EI) mass spectra. Furthermore, 24-epimers of BL and CS are also completely separated by capillary GC [15, 16]. In the case of 2-deoxy BRs such as teasterone and typhasterol, which have a vicinal diol in the side chain and single hydroxyl group on the A-ring, the diol moiety is first converted to the methaneboronate and then the remaining 3-hydroxyl group is trimethylsilylated with an appropriate silylating reagent to give a methaneboronate–trimethylsilyl (MB–TMS) derivative [56] (Scheme 128.2). The MB–TMS derivatives of teasterone, typhasterol, and their 28-homo and 28-nor analogs are well separated by capillary GC [56]. These derivatives have limit of detection (LOD) values in the nanogram range for full-scan EI-MS analyses and subnanogram LOD values for analyses by EI-GC in SIM mode.

5.3 High-Performance Liquid Chromatography (HPLC)

HPLC is one of the most frequently used analytical methods for the separation and analysis of BRs [57]. Because BRs have no suitable chromophore for detection, they are derivatized with prelabeling reagents that are suitable for use with ultraviolet (UV), fluorometric, or electrochemical detectors. Several boronic acid reagents that react with the vicinal diol groups of BRs have been used as prelabeling reagents, including naphthaleneboronic acid for UV detection [58], 9-phenanthreneboronic acid [59], 1-cyanoisoindole-2-m-phenylboronic acid [60] and (dansylamino)phenylboronic acid [61] for fluorometric detection, and ferroceneboronic acid for electrochemical detection [62]. The limits of detection for these methods range from 25 to 100 pg per injection, depending on the derivative used. The most effective of these reagents seems to be (dansylamino) phenylboronic acid because its chromophore can be detected at longer wavelength (excitation 345 nm/emission 515 nm) than other boronates, and so its signal is less susceptible to interference from matrix contaminants. The derivatized BRs are effectively separated by HPLC using ODS columns with acetonitrile–water as the mobile phase.



Scheme 128.2 Conversion of brassinolide to its bismethaneboronate derivative and teasterone to its methaneboronate–trimethylsilyl ether

Gamoh et al. [63] developed a HPLC method based on a combination of precolumn labeling and postcolumn fluorescence detection that can be used to separate and detect 24-epimers of BL and CS. Related reverse-phase HPLC methods have also been used successfully to analyze BRs in the pollen of broad bean, corn, sunflower, buckwheat, and orange [64–66].

5.4 Liquid Chromatography–Mass Spectrometry (LC–MS)

Microanalytical LC–MS methods for the analysis of BR-derived boronates have also been developed, using either atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). APCI-based LC–MS has been used to analyze naphthaleneboronate derivatives of BRs; in this case, optimal results were obtained by using a reverse-phase HPLC acetonitrile–water gradient to elute a C18 column [67]. Typical ions observed in the positive-ion spectra of the naphthaleneboronates included a pseudomolecular ion $[M + H]^+$ and a fragment ion $[M + H\text{-}H_2O]^+$. The most abundant ion from the fragmentation of BL derivatives is the parent ion, while the $[M + H\text{-}H_2O]^+$ ion is the most abundant in the mass spectra of CS, teasterone and typhasterol. Full-scan mass spectra were readily obtained from 400 ng of free BRs, whereas the limit of detection in SIM mode was around 2 ng. The most

effective mobile phase for naphthaleneboronates was found to be a mixture of acetonitrile and water (9:1) containing 0.5 % HCOOH. Svatoš et al. [68] reported a highly sensitive and selective LC–(ESI)-MS method that was developed for analyzing BRs in plant extracts. The method involves using a microbore C18 column (1.0 mm) in conjunction with chemical derivatization of free BRs to dansyl-3-aminophenylboronates. This gives it a much lower limit of detection than can be obtained with previous analytical methods: its limit of detection for derivatized BRs in selected ion-monitoring (SIM) mode is 125 attomoles. The practical utility of the method was demonstrated in *Arabidopsis thaliana* plants that were fed with a deuterium-labeled precursor of CS and converted it to BL [68]. A highly sensitive electrospray ionization mass spectrometry (MS) technique (LOD 50 fmol) has been developed and used for direct analysis of natural brassinosteroids in different plant tissues [12]. This method was successfully used to detect and quantify brassinolide and castasterone in rape pollen and seedlings of *Daucus carota, Phaseolus vulgaris*, and *A. thaliana* [12]. Despite these advances, there is considerable scope for further improvements upon existing analytical methods and for reducing their LODs by using capillary LC systems coupled to a nano-ESI source. Additional improvements could be made by using ultra-performance liquid chromatography (UPLC) or by developing brassinosteroid-specific immunoaffinity chromatography techniques for the analysis of natural plant samples.

6 Biological and Pharmacological Activities

The best known and the most widely studied biological effect of BRs is their ability to stimulate plant growth in a variety of systems such as whole plants, excised segments, cuttings, and seedlings.

Brassinosteroids have been observed to stimulate seedling elongation in many assay systems, including normal and dwarf pea epicotyls, dwarf bean apical segments, mung bean epicotyls, and cucumber and sunflower hypocotyls [69]. In general, BRs stimulate elongation in the light but not in the dark [70]. Kamuro and Inada [71] examined the effects of light on the growth-promoting effects of BL using mung bean (*Vigna radiata)* epicotyls. Cuttings with attached cotyledons underwent epicotyl elongation in the dark and also under monochromatic blue light (452 nm) or far-red light (722 nm). BL did not promote epicotyl growth under any of these conditions. Epicotyl growth was retarded by white light (400–700 nm) and monochromatic red light (660 nm), and the growth-promoting effects of BL were clearly observed under these light regimes. Cuttings that were alternately irradiated with red and far-red light exhibited retarded epicotyl growth if the treatment ended with exposure to red light; BL treatment overcame this retardation. These results indicated that the effects of BRs are related to phytochrome-mediated growth regulation.

Brassinosteroids have also been observed to inhibit adventitious root development in mung bean hypocotyls [72]. Subsequent work reviewed by Roddick et al. [73] indicated that this inhibition might not be a primary action of BRs and may

instead be due to differences in the optimal concentration of BRs for adventitious root development and shoot elongation. When soybean (*Glycine max* L.) hypocotyl segments were produced under photoperiods of 16-h light and 8-h darkness, adventitious roots were induced by treatment with 24-epiBL at the very low concentration of 0.0001 μg/ml during the dark periods. There is also evidence that BRs may regulate root hair development: Kappusamy et al. [74] reported that the levels and patterns of expression for two master epidermal patterning regulators (WEREWOLF and GLABRA2) are regulated by BRs and that plants with BR-related mutations exhibit abnormal root hair development.

The effect of BRs on cell division is somewhat controversial. In carrot (*Daucus carota* L.) and tobacco cell cultures, BRs induced cell enlargement without any effect on cell division [75, 76]. In a culture of *Zinnia elegans* L. mesophyll cells, the inhibition of endogenous GA_3 and BR biosynthesis by treatment with uniconazole retarded tracheid differentiation, which could be restored by treatment with BL or 28-homoBL (but not by GA_3) [77]. BL is essential for entry into the final stage of tracheary element differentiation, where secondary wall formation and cell death occur [78]. Using tuber explants of *Helianthus tuberosus*, Clouse and Zurek [79] also showed that BL stimulates xylem differentiation. Under normal conditions, xylem differentiation occurred only after 3 or 4 days' incubation of the explant on xylem-inducing media. However, when BL was added at a concentration of $6.8.10^{-9}$ M, significant xylem differentiation was observed after 24 h. This evidence suggests that BRs may influence cytodifferentiation in plants [80]. While investigating the potential role of BRs in regulating cell division, Hu et al. [46] found that treating *Arabidopsis* seedlings with 24-epiBL can upregulate the expression of CycD3, a D-type plant cyclin gene that may promote cell division.

Many studies have suggested that BRs play essential roles in responding to various stresses such as abnormal temperatures, drought, high osmotic pressure, and pathogen attacks [81, 82]. The occurrence of cross-talk between BRs and stress-responsive hormones such as abscisic acid, jasmonic acid, and ethylene is consistent with the suggestion that BRs play an important role in plant stress responses [83].

The ability of BRs to enhance plant resistance to various external stresses has been investigated with a view to finding applications for them in agriculture. Because temperature changes are likely to occur more rapidly than other stress-causing factors in nature, many studies in this area have focused on temperature stress. Maize seedlings are highly sensitive to chilling stress during germination and the early stages of growth. Treatment with BRs promoted growth recovery in maize seedlings that had previously been chilled. BRs also promoted the greening of etiolated leaves at low temperature in light [84]. Schilling et al. [85] examined the effects of 28-homoBL on sugar beet (cv. Ponemo) under drought stress. Treatment with 28-homoBL caused an increase in taproot mass, sucrose content, and sucrose yield under drought stress; no such effect was observed under non-stress conditions. Brassinosteroids have also been shown to activate total protein synthesis in wheat leaves under high-temperature stress. BR-treated leaves that were maintained at 43 °C exhibited protein synthesis levels similar to those observed at 23 °C.

However, in untreated leaves maintained at 43 °C, protein synthesis decreased by a factor of 2.5 relative to control samples at 23 °C [86]. In plant cells under heat stress, small heat shock proteins aggregate to produce highly ordered cytoplasmic complexes known as heat shock granules (HSGs). In wheat leaf cells, HSGs aggregate further to form small clusters. The average number of HSGs in the clusters and the average cluster size were both higher in BR-treated leaves than in untreated leaves [86].

The ability of BRs to enhance plant resistance to infection by fungal pathogens has also been investigated [87]. It was demonstrated that potato plants sprayed with BR solution were less prone to infection by *Phytophthora infestans*. The increase of resistance in BR-treated potato tubers was associated with elevated abscisic acid and ethylene production as well as increased levels of phenolic and terpenoid substances. In some investigations, BRs proved to be more effective than standard fungicides at protecting against fungal infection. However, certain concentrations of BRs and treatment with BRs at certain plant developmental stages can stimulate fungal growth and disease progression. As such, it would be necessary to carefully consider the timing of BR application, the concentration applied, and the method of application when developing a potential BR-based fungicidal treatment [88].

The brassinosteroids are essential for many growth and development processes in plants. However, relatively little is known about the effects of BRs and their synthetic analogs on animal cells [89]. It has been reported that cultured hybridoma mouse cells treated with 24-epiBL exhibit certain interesting effects, including an increase in the mitochondrial membrane potential, a decrease in the abundance of intracellular antibodies, an increase in the fraction of the cells in the G_0/G_1 phase, and a corresponding decrease in the proportion of cells in S phase. Moreover, treatment with 24-epiBL at concentrations of 10^{-13} and 10^{-12} mol/l significantly increased the density of viable cells relative to controls [90].

It has recently been reported that the natural BRs 24-epiBL and 28-homoCS have significant effects on the growth and viability of various normal and cancerous cell lines [23]. 28-HomoCS and 24-epiBL were shown to affect the viability of human cancer cell lines of various histopathological origins, but not human fibroblasts (BJ). Cell lines tested included the T-lymphoblastic leukemia CEM, breast carcinoma MCF7, lung carcinoma A-549, chronic myeloid leukemia K562, multiple myeloma RPMI 8226, cervical carcinoma HeLa, malignant melanoma G361, and osteosarcoma HOS. Treatment with 28-homoCS and 24-epiBL resulted in potent, dose-dependent reductions in the viability of CEM and RPMI 8226 cells, although the severity of these effects differed between the two compounds.

Estrogen- and androgen-sensitive and -insensitive breast and prostate cancer cell lines were shown to respond differently to treatment with natural BRs. Most breast cancers consist of a mixture of estrogen-sensitive and estrogen-insensitive cells, and it seems that it is necessary to eliminate both cell types in order to control breast cancer. Hormone-sensitive cell lines were more susceptible to BR treatment. This finding may indicate that natural BRs are capable of modulating the steroid receptor response in human cancer cells. Natural BRs were found to be cytotoxic to cancer cells but not to untransformed human fibroblasts, suggesting that they induce

different responses in cancerous and normal cells. As such, these plant hormones may be useful leads for developing novel anticancer drugs [23]. There is also evidence that brassinosteroids may perturb cell cycling in breast and prostate cancer cell lines. Using flow cytometry, it was shown that treatment with 28-homoCS and 24-epiBL [104] induced a blockage in the G_1 phase of the cell cycle in breast and prostate cell lines, with concomitant reductions in the percentage of cells in the S phase of the cell cycle [23]. In the MCF7 breast cancer cell model (which is the most widely studied experimental system in this context), this response mirrors the typical response to antiestrogens, which also reduce the proportion of cells that are actively synthesizing DNA (i.e., cells that are in the S phase) and concomitantly increase the proportion of cells in the G_0/G_1 phase [91].

Angiogenesis, i.e., the growth of new blood vessels in animals, is essential for organ growth [92] and for the growth of solid tumors and metastasis [93]. Potent angiogenic inhibitors capable of blocking tumor growth are thus promising leads for the development of a new generation of anticancer drugs [94]. Recently, several steroids (i.e., 2-methoxyestradiol, progestin, medroxyprogesterone acetate, and glucocorticoids such as dexamethasone and cortisone) have been shown to have antiangiogenic activity [95]. Until recently, nothing was known about the effects of natural BRs on angiogenesis or other processes in endothelial cells. A study was therefore conducted to investigate the effects of naturally occurring BRs and their synthetic analogs on cell proliferation and cycling in human microvascular endothelial or umbilical vein endothelial cells, with a particular emphasis on the migration and formation of tubes within these cells [89]. Although the antiangiogenic activity of natural BRs was weak, it was found that the synthetic BR-derivative cholestanone significantly inhibited angiogenesis in endothelial cells in vitro. At a concentration of 30 μM, cholestanone reduced migration to 38 % relative to untreated cells and decreased the number of tubes by 34 % relative to the control treatment. As such, the BR-derivative cholestanone was a significantly more potent inhibitor of migration and tube formation than natural brassinosteroids [96].

Brassinosteroids seem to exert their effects on growth and the cell cycle effects via the cell cycle machinery and apoptosis and may thus be useful leads in the development of novel agents for managing cancer. The antiangiogenic effects of BRs may also prove useful for controlling tumor growth and metastasis, potentially paving the way for the development of multiple novel phytohormone-derived anticancer drugs [89, 96].

7 Agronomical Uses

BRs have strong effects on plant growth and possess a unique combination of physiological actions that have considerable potential for practical applications. Their potential economic value as agents for increasing crop yields has been discussed previously [97]. Their applications in agriculture and horticulture would be based on their ability to stimulate plant growth and enhance plant

Table 128.1 Selected results from brassinosteroid field trials

Plant	BR applied, dose, mode of treatment	Yield enhancement (%)	References
Wheat	24-epiBL, 4 mg/ha, spraying at flowering	16	[100]
Corn	24-epiBL, 50 mg/ha, spraying at emergence of tassel	10	[101]
Soybean	24-epiBL, 50 mg/ha, spraying at flowering	5	[100]
Potato	24-epiBL, 20 mg/ha, spraying at budding	18	[102]
Tomato	24-epiBL, 25 mg/ha, spraying at flowering	33	[103]
Sugar beet	24-epiBL, 50 mg/ha, spraying at 2–3 leaves	7	[102]

resistance to various external stresses. These effects could make it possible or attractive to grow crops under otherwise-unfavorable conditions such as in areas that have high salinity, are undergoing drought, or have an insufficient nutrient supply. Moreover, BRs have some specific properties that could be very valuable in practical applications [87]. These include:

1. BRs are natural products and are ubiquitous in the plant kingdom. As such, they are a long-standing component of the food chains of men and mammals; the biosynthetic and metabolic pathways of plants and mammals have coevolved over a long period of time and are heavily interconnected.
2. Plants respond to very small doses of BRs (5–50 mg/ha) that are comparable to those that occur naturally.
3. As plant growth-promoting substances, BRs have a broad spectrum of stimulatory and protective activities, all of which should have positive effects on the quantity and quality of crop yields.
4. BRs increase plant resistance to phytopathogens and can be used as substitutes (total or partial) for some traditional pesticides. Their use in this way would reduce the need to use pesticides that interact unfavorably with the environment.
5. BRs can be easily applied to plants and seeds using existing equipment and technologies.

The most commonly used BR in field trials is 24-epibrassinolide because of its high biological activity and relatively simple and synthesis. Treatment with 24-epiBL has been reported to increase yields of wheat, tobacco, rape, orange, grape, and sugar beet [98, 99]. Moreover, the application of 28-homoBL and 24-epiBL to potato plants at a dosage of 10^{-20} mg.ha^{-1} increased yields by 20 % and improved the quality of the crop by decreasing its nitrate content and increasing its content of starch and vitamin C [87]. Some other selected results from BR trials with different agricultural plants are presented in Table 128.1.

The influence of BRs on development and crop yield in different crop plants such as cereals, legumes, vegetables, and fruits has been tested under both laboratory and field conditions. For large-scale field applications, there are two viable methods for applying BRs: soaking seeds and foliar spraying. The performance of these two methods has been investigated in depth; the results obtained with foliar

spraying proved to be very dependent on the stage in the plants' development at which it was applied. In general, the best results were obtained when young plants were treated [87].

8 Conclusion

Brassinosteroids are now firmly established as essential regulators of plant growth and development that affect a broad spectrum of processes at the molecular, cellular, and physiological levels. BRs are natural nontoxic, non-genotoxic, biosafe, and eco-friendly plant products and can therefore be used in agriculture and horticulture to improve the growth, yields, quality, and tolerance of various plants to biotic and abiotic stress.

Over the past decade, genetic screening has been widely used for identifying and characterizing major components and genes involved in the BR signaling pathway. However, the molecular mechanisms of these processes remain largely unknown. As such, there is a need for additional research to understand the mechanisms by which BRs affect plant and animal cells on the molecular level.

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