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Chemistry of Abscisic Acid, Abscisic Acid Catabolites and Analogs

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Abstract

Recently there have been breakthroughs on a number of fronts in abscisic acid (ABA) biology research that have advanced the field significantly, including discovery of genes involved in ABA metabolism, along with progress in understanding of ABA signaling (Finkelstein and others 2002; Kushiro and others 2004; Lim and others 2005; Saito and others 2004). At the same time, the chemistry of ABA has advanced. New analytical methods have been developed for profiling ABA and catabolites (Ross and others 2004; Zaharia and others 2005). Novel bioactive catabolites have been discovered from feeding studies with deuterated ABA and catabolites (Zaharia and others 2004; Zhou and others 2004). This review covers recent advances and prospects in natural products chemistry, analysis of ABA catabolism, and applications of ABA analogs for biochemical studies and horticultural uses.

Key words: ABA; abscisic acid; ABA metabolism; ABA analogs; ABA binding proteins; Hormone profiling; Structure–activity studies

Introduction

ABA Metabolism

The concentrations of biologically active abscisic acid (ABA) and metabolites in plant tissues are the result of a combination of transport, biosynthesis, and metabolism. In plants, ABA can be metabolized through conjugation and oxidation at different positions, the preference for one pathway or the other depending on the plant species, plant part, developmental stage, or biological process involved (Cutler and Krochko 1999; Zeevaart 1999; Oritani and Kiyota 2003).

The principal oxidative pathway of natural ABA (**1a**, (+)-*S*-enantiomer, Figure 1) has been well studied (Zeevaart 1999; Oritani and Kiyota 2003). This oxidation of ABA is mediated by cytochrome P-450 monooxygenases (Krochko and others 1998; Kushiro and others 2004; Saito and others 2004), occurs through hydroxylation of the 8′-methyl group resulting in 8′-hydroxyABA (**2**), which rearranges to

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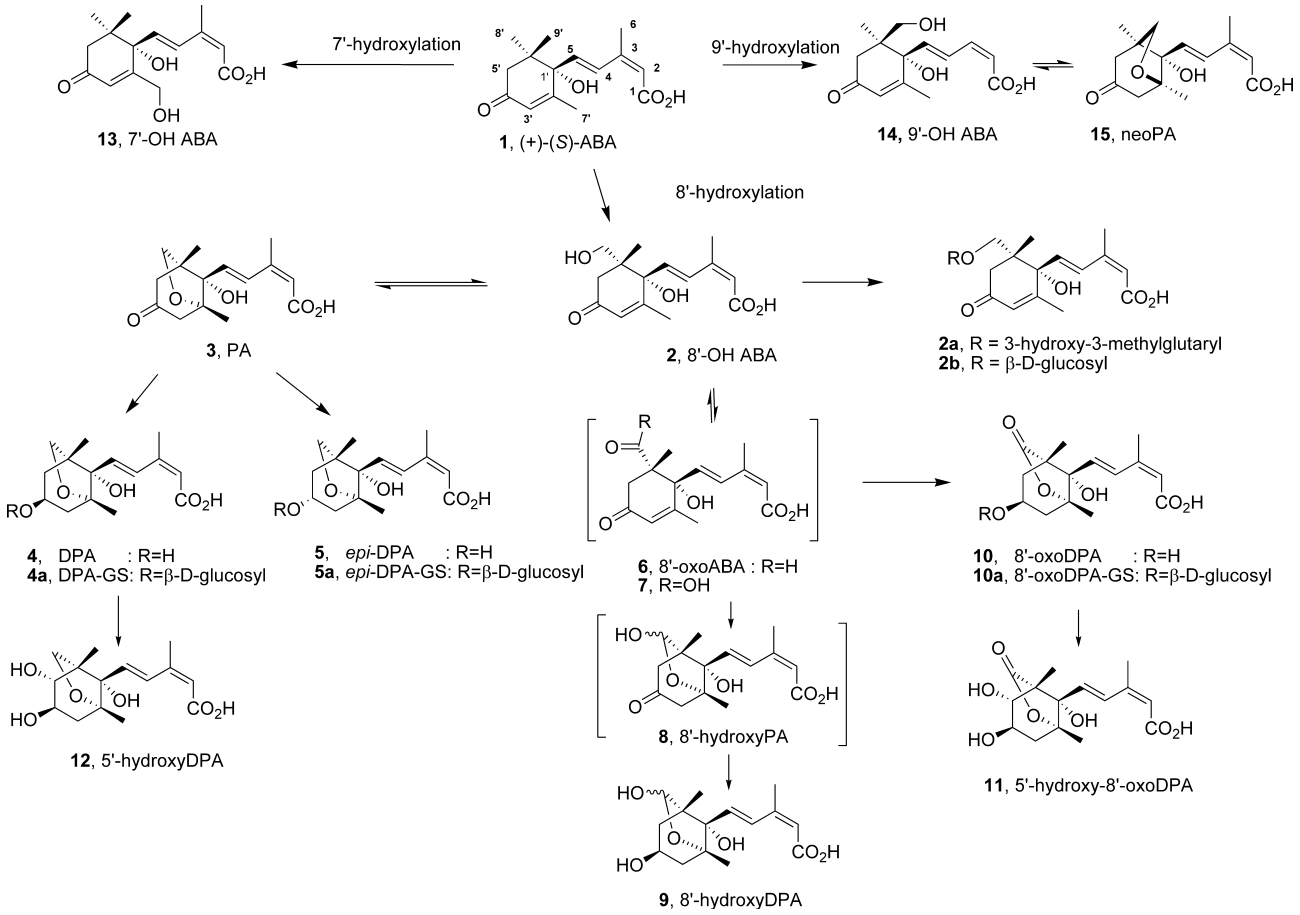

Figure 1. Oxidative pathways of metabolism of ABA.

phaseic acid (**3,** PA). Reduction of the 4′-carbonyl group of PA gives major dihydrophaseic acid (**4**, DPA) and minor *epi*-dihydrophaseic acid (**5**, *epi*-DPA).

Recently Zaharia and others (2004) have reported new metabolism products of ABA arising from further oxidation at the 8′-position, isolated from corn cell suspension cultures fed ABA. These researchers established the involvement of an aldehyde-type intermediate (**6**, 8′-oxoABA) obtained through oxidation of 8′ hydroxyABA (as shown in Figure 1). This pathway appears to be significant in both cell cultures and plants, and it appears that at least 30% of the PA pool has been further oxidized and then reduced. This calculation is an underestimate, based on isotope effects. The ''futile cycle'' was deduced from the observation that the PA resulted from the metabolism of a C-8′ deuterium-labeled ABA contained in position H-8′ *exo* a mixture of 83% D and 17% H, suggesting a stereoselective reduction of the putative aldehyde intermediate. Feeding experiments of the synthetic intermediate to the corn cell suspension cultures showed that the aldehyde was reduced to 8′-hydroxyABA, which cyclized to PA. Also, isolation of deuterium-labeled 8′-hydroxyPA (**8**), 8′-hydroxyDPA (**9**), and 8′-oxoDPA (**10**) as ABA metabolism products supported their rationale (Figure 1), as the catabolites can be obtained from **6** either by hydration and cyclization (for **8**) or through oxidation to the acid (**7**), followed by cyclization/reduction to **10**. However, it was not clear what the substrates for the formation of these compounds were, because the aldehyde (**6**), the PA (**3**), or the DPA (**4**) could be further oxidized at C-8′. For example, 8′-oxoDPA (**10**) could arise from the aldehyde (**6**) via 8′-oxoPA or from DPA (**4**) via 8′-hydroxyDPA (**9**), suggesting that the C-8′ oxidation pathway could be more complex than previously thought. The authors speculate that these further oxidative processes could represent a mechanism for enhancing ABA catabolism. The first catabolite, 8′-hydroxyABA, has been found to have hormonal activity (Zou and others 1995). The role in ABA signaling of the aldehyde and the cycle is unknown.

Moreover, the isolation of compounds **10**, **10a**, **11,** and **12** from plums (*Prunus domestica* L.) was

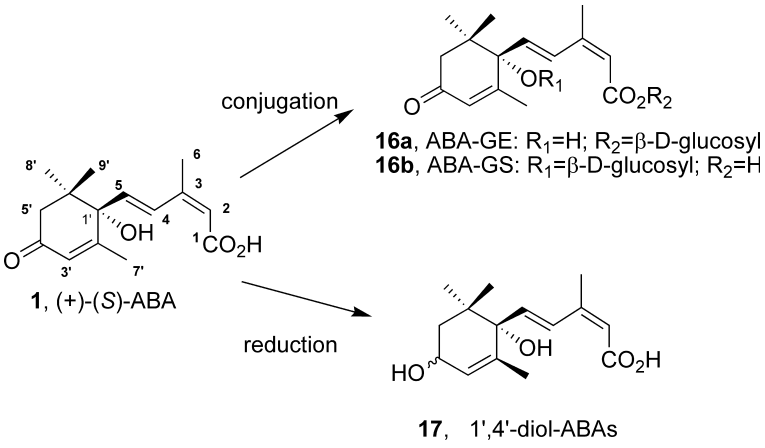

Figure 2. Other pathways of metabolism of ABA.

reported (Kikuzaki and others 2004). These compounds were isolated from an ethanol extract with antioxidative activity and were not proven to be derived directly from ABA. Their structure supported the proposed metabolic pathway in which further oxidation at C-8′ occurred. Together with ABA, **10–12** exhibited antioxidant activity.

A second oxidation pathway, apparently minor, occurs through hydroxylation of the 7′-methyl group of ABA (Zeevaart 1999; Oritani and Kiyota 2003) resulting in 7′-hydroxyABA (**13**). This compound also has hormonal activity in ABA assays (Hill and others 1995; Taylor and others unpublished). Catabolites of 7′-hydroxyABA have not yet been identified.

Recently, Zhou and others (2004) reported the discovery of a third oxidative pathway occurring in *Brassica napus* siliques. This finding resulted from the mass spectrometric (MS) analysis of deuterated oxidized metabolites obtained from feeding (+)-ABA analogs deuterated at C-8′ and at both C-8′ and C-9′. Analysis of the products indicated that hydroxylation of ABA had occurred at the 9′-methyl group of ABA, as well as at the 7′- and 8′-methyl groups. The new compound isolated from plant extract was identified as the closed form of 9′-hydroxyABA (**14**), which they named *neo*phaseic acid (**15**, neoPA). By analogy with 8′-hydroxylation, the authors proposed that 9′-hydroxyABA (**14**, open form) would be the first product of 9′-oxidation, which can cyclize to neoPA. NeoPA (**15**) was also detected in oranges (*Citrus sinensis*), tomato (*Lycopersicon esculentum*), *Arabidopsis* plants, and chickpea (*Cicer arietinum*), as well as in drought-stressed barley (*Hordeum vulgare*) and *Brassica napus* seedlings. Levels of **15** were higher in immature seeds, and disappearance of **15** at late stages of seed and pericarp development suggested further metabolism. The open form (9′-hydroxyABA), but not neoPA, showed ABA-like bioactivity in inhibiting *Arabidopsis* seed germination and in inducing lipid-related gene expression in *B. napus* microspore-derived embryos. The biological significance of 9′-hydroxyABA is yet to be determined.

Both ABA and metabolites of the major oxidative pathway can be further conjugated, mainly with glucose (Zeevaart 1999; Oritani and Kiyota 2003). Thus, ABA can form the glucose ester at C-1 (**16a,** ABAGE) or the glucoside at C-1′ (**16b**), as shown in Figure 2. The fate of ABAGE is unknown. In lettuce seed germination, ABAGE levels were found to rise and then fall in germinating seedlings (Chiwocha and others 2003). Dihydrophaseic acid can be further metabolized to its glucose conjugate **4a**. Also, Ramos and others (2004) reported the isolation of two new glucose conjugates from avocado seeds (*Persea americana*), namely *epi*-DPA-β-D-glucoside (**5a**) and 8′-hydroxyABA-β-D-glucoside (**2b**). Although not surprising, the existence of such conjugated forms of the ABA metabolism products raises the question of their role in the pathway, whether they represent storage or a transport form of that particular metabolite. Although minor, the reductive pathway of ABA to rather unstable 1′,4′-diol-ABAs (**17**, Figure 2) has also been shown (Zeevaart 1999; Oritani and Kiyota 2003).

Overall, considerable progress has been made toward finding new ABA metabolites. However, the catabolism scheme is far from being complete, because further metabolism of DPA, 7′- and 9′-hydroxyABA is yet to be discovered. Moreover, a better understanding of the activity and role of these metabolites in different ABA-related biological processes occurring in plants is needed.

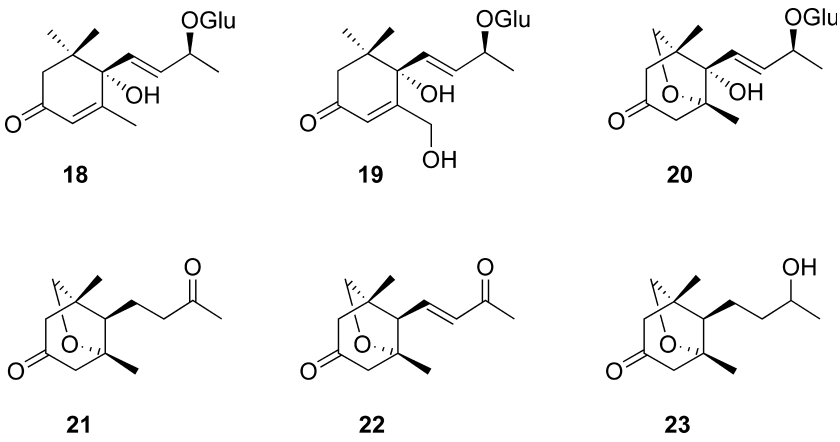

Figure 3. Abscisic acid–related compounds.

ABA-Related Compounds

Recently, the isolation of several ABA and PA-like compounds has been reported. Thus, Calis and others (2002) reported the isolation of chorchoionoside (**18**), as well as spionoside A (**19**) and B (**20**) (Figure 3), from the methanolic extract of mature fruits of *Capparis spinosa*. Their structure resembles that of ABA, 7′-hydroxyABA, and PA, except for the ABA side-chain. The apocarotenoids annuionones A (**21**), B (**22**), and E (**23**) provide a similar example. These substance were isolated by Macias and others (2004) from fresh leaves of *Helianthus annuus* L. (sunflower), whose revised structure shows the presence of the bicyclic ether structure of PA, except for the side-chain portion of the molecule. However, the structural resemblance to ABA does not qualify these structures as ABA metabolites. Feeding studies with labeled ABA or ABA precursors would establish whether such compounds are truly ABA catabolism products in those particular plant systems or would confirm that they have similar carotenoid precursors.

In-depth knowledge of ABA catabolism brings better understanding about how the ABA pool is being modified under different physiological conditions and biological systems. Deuterium-labeled ABA catabolites of high specific incorporation are prepared either by isolation from feeding labeled ABA to plant cells or by chemical synthesis. Such labeled compounds are to be used either as internal standards in quantitative analysis using various methods or as feeding substrates to monitor their further metabolism. Deuterated ABA analogs bearing stable isotope label at non-exchangeable positions have been used as starting materials for standards of ABA metabolites. Thus, Abrams and others (2003) reported the synthesis of ABA analogs with greater than 95% incorporation of deuterium atoms at C-4 and/or C-5 of the side chain of the molecule, introduced by deuteride or hydride reduction of the intermediate propargylic alcohol. Three deuterium atoms have also been introduced at the C-8′ position by conjugate addition of a D-containing Grignard reagent to the cyclohexadienone intermediate (Abrams and others 2003). These deuterated analogs have been used as substrates for metabolism studies to prepare labeled PA, DPA, and DPA-GS, all with high incorporation of label at the 4, 5 or 8′-positions, as reported by Zaharia and others (2004). With their aid, the existence of the advanced oxidative metabolism at the C-8′ methyl group was proven, as previously discussed (Zaharia and others 2004).

Recently, Hirai and others (2003) reported the preparation of 7′-deuterium-labeled PA with a deuterium content of 86%. The introduction of the deuterium label was achieved by treatment of non-labeled PA with NaOD for 26 days, followed by reverse D-H exchange through NaOH treatment of the labeled PA (Hirai and others 2003). 7′-Deuterium-labeled dihydrophaseic acids were obtained by reduction of 7′-deuterium-labeled PA with $NaBH_4$. A different method (Zaharia and others 2005) reported the preparation of deuterium-labeled ABA metabolites as biotransformation products of labeled ABA by maize cell suspension cultures. High incorporation of label at key positions of the starting material was achieved through chemical synthesis, suitable for large-scale preparation (Abrams and others 2003). Thus, [7′,7′,7′-d_3]-PA, [7′,7′,7′-d_3]-DPA, and [5,8′,8′,8′-d_4]-7′-hydroxyABA were obtained. Moreover, the preparation of non-labeled and [4,5,8′,8′,8′-d_5]-ABAGE using an improved synthetic method was presented.

To complement plant genomic studies, new analytical methods and internal standards are being developed for quantitation of phytohormones

involved in the regulation of important physiological processes in plants, including ABA and catabolites (Dewitte and Van Onckelen 2001; Gomez-Cadenas and others 2002; Muller and others 2002; Birkemeyer and others 2003; Schmelz and others 2003, 2004; Ross and others 2004). Although rigorous methods for measuring the ABA content in plant tissue have been developed, such methods for ABA catabolite quantitation are limited by a lack of internal standards. Early analytical methods employed to measure levels of ABA metabolites (PA, DPA, *epi*-DPA, ABAGE) consist of gas chromatography with electron capture detection (GC-ECD) (Harrison and Walton 1975; Zeevaart and Milborrow 1976) and GC coupled with mass spectrometry (GC-MS), methods that require partial purification and derivatization of the metabolites. The techniques used for quantitative determination of conjugated ABA are based on the analysis of ABA released by alkaline hydrolysis of the conjugate. However, Boyer and Zeevaart (1982) developed a method in which ABAGE is measured by GC-ECD as its tetraacetate derivative (Boyer and Zeevaart 1982). GC-MS with selected ion monitoring (GC-MS-SIM) (Netting and others 1982) and multiple-ion monitoring (GC-MS-MIM) mode (Neill and others 1983), using isotopically labeled internal standards was frequently used for ABAGE quantification. Radioimmunoassays using monoclonal (Perata and others 1990) and polyclonal (Weiler 1979; Weiler 1980) antibodies have also been used for quantitation of conjugated ABA. Hogge and others (1993) reported the quantification of ABAGE using liquid chromatography-continuous flow secondary-ion mass spectrometry with reaction monitoring, whereas Schneider and others (1997) quantified ABAGE by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS-MS).

Recent methods of hormone metabolic profiling in plant tissues use reversed-phase high performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) (Zhou and others 2003; Ross and others 2004). Ross and others (2004) have developed a sensitive method suitable for polar compounds, using LC-MS with multiple reaction monitoring (MRM) in negative ion ESI for specific detection and accurate quantification of endogenous and supplied ABA in plant tissue. Isotopically labeled ABA analogs were used not only as feeding material in metabolism studies and as internal standards, but also for the elucidation of the fragmentation pathways through LC-MS. The fragmentation of the anion of ABA is more complex than simple cleavage of the side chain. The side chain is lost as a neutral fragment of even mass. The protons of the alcohol and C-4 carbon exchange can be explained by invoking a cyclic intermediate (Ross and others 2004). These fragmentation studies allowed the selection of the product ions most suitable for MRM, transitions that are distinct from the labeled compounds (internal standards and feeding material) and their counterparts produced endogenously in the plant. This method has been adapted for simultaneous profiling of ABA metabolites and other plant hormones. At present, this method uses [7′,7′,7′-d_3]-PA, [7′,7′,7′-d_3]-DPA, [5,8′,8′,8′-d_4]-7′-hydroxyABA, and [4,5,8′,8′,8′-d_5]-ABAGE of high purity and high isotopic incorporation at specific positions as internal standards. It has become widely used for the ABA metabolites analysis in seed development and germination, namely lettuce seeds (Chiwocha and others 2003), western white pine seeds (Feurtado and others 2004), and *Brassica napus* siliques (Zhou and others 2004), as well as for evaluating the role of ABA in controlling developmental switching in *Marsilea quadrifolia* (Lin and others 2005).

ABA Analogs

With recent advances in genomics and dissection of ABA signaling in plants, there is a greater opportunity to employ analogs to address specific biochemical questions on the mode of action of ABA (Finkelstein and others 2002). Since the discovery of the structure of ABA in the early 1960s, chemists have synthesized hundreds of ABA analogs and metabolites of the plant hormone for structure/activity studies to study ABA mode of action and to identify ABA receptors. These synthesized forms have agricultural and horticultural application as plant growth regulators and they serve to probe the roles of ABA metabolites in plant processes in which ABA is known to be involved.

Reviews of structure/activity studies (Walton 1983; Todoroki and Hirai 2002; Oritani and Kiyota 2003) have attempted to compile diverse experimental results of studies of how the ABA molecule is perceived by plants, with some success, despite the complexity of the problem. Activity results have generally been obtained from physiological studies on biochemically complex processes such as germination, growth inhibition, and transpiration, in which the analogs have been tested in small sets and as racemic mixtures. Studies conducted with optically pure analogs and gene expression assays have provided some clear insight into the functioning of the plant hormone in specific instances.

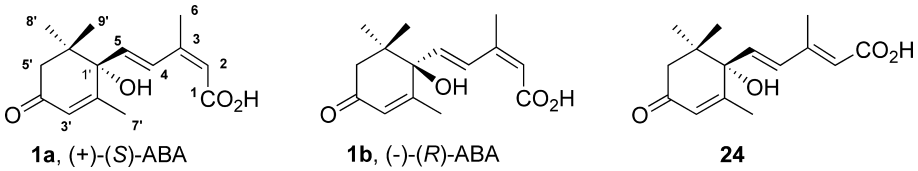

Figure 4. *S*-ABA, *R*-ABA and 2-*trans*-ABA.

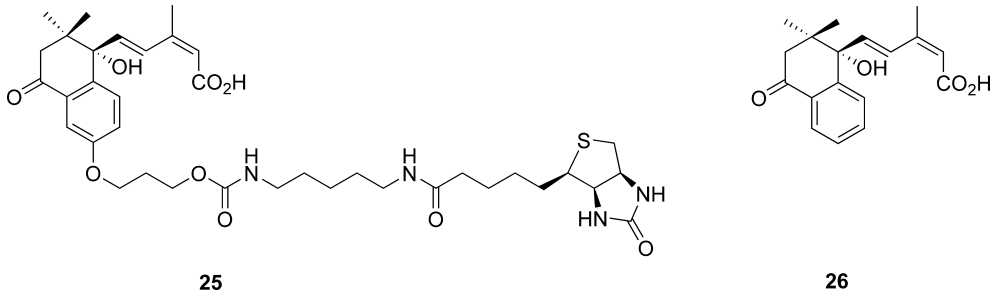

Figure 5. Abscisic acid analogs—probes for identifying ABA-binding proteins.

As more ABA binding proteins become known, it will be possible to probe binding pockets with structurally defined analogs. This will enable study of the shape ABA adopts in an active site and design analogs to fit it into some but not other binding proteins.

Here we suggest, given the present state of knowledge, specific analogs that can be useful for biochemical studies and caution about others that can give confounding results. We also discuss the prospects for analogs for plant growth regulation applications.

S-ABA and R-ABA

Whenever possible, to study the action of ABA in biochemical studies, we recommend the use of the natural hormone *S*-ABA. The cost of *S*-ABA has decreased with commercial production of the hormone by fermentation. Plants synthesize only *S*-ABA (**1a)**, and *R*-ABA (**1b)** (Figure 4), the mirror-image form, has not been reported in nature. In physiological assays the effects of *R*-ABA and racemic ABA (which is a 1:1 mixture of the mirror-image forms), are not necessarily equivalent to the effects of *S*-ABA, the natural plant hormone. The rates of metabolism in plant cells are different, and the persistence of the *R*-ABA is greater (Abrams and others 1989). The effects of the two forms can be different in inducing gene expression (Walker-Simmons and others 1992) and physiological responses. *R*-ABA has little ABA-activity in stomatal aperture assays (Walton 1983) and in inducing expression of genes involved in lipid synthesis. In many assays, however, *R*-ABA shows strong activity, such as inhibiting growth in wheat embryos (Walker-Simmons and others 1992). The two forms are metabolized by plant enzymes to different products, which is significant, as some of the catabolites of *S*-ABA also have hormonal activity (Zou and others 1995; Zhou and others 2004). Supplied *R*-ABA can induce biosynthesis of *S*-ABA in plant tissues, as was found when deuterated *R*-ABA was supplied to *Marsilea quadrifolia* plants, and levels of *S*-ABA and its metabolites were found to increase (Lin and others 2005). The differences in activity between the two ABA forms was exploited by McCourt and coworkers in a genetic screen to find mutants altered in ABA signaling (Nambara and others 2002).

ABA Analogs—Probes for Identifying ABA-binding Proteins

Affinity methods employing tethered ABA analogs have potential as reagents for isolating ABA-binding proteins. Direct photochemical approaches toward identifying ABA receptors have been unsuccessful to date. Abscisic acid probes tethered either through the C-1 carboxyl group or the C-4′ ketone have been used to isolate plant proteins that bind ABA (Todoroki and Hirai 2002). The carboxyl group and the ketone group have both been shown to be required for activity, and probes derived through alteration of either functional group may not provide the correct presentation of the ABA molecule for binding to ABA binding proteins, particularly receptors (Pedron and others 1998; Zhang and others 2002; Yamazaki 2003). Our approach to solving this problem was to design a probe, **25** (Figure 5), attaching a tether to an aromatic ring

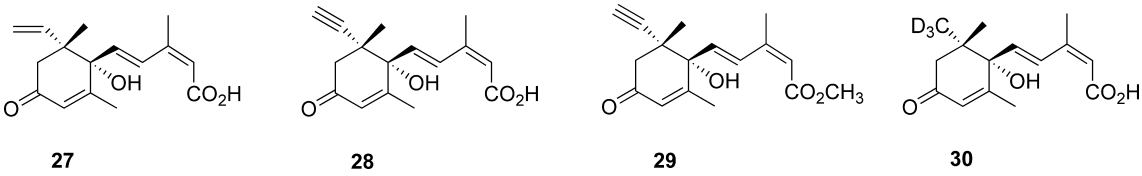

Figure 6. Abscisic acid analogs—persistent ABAs.

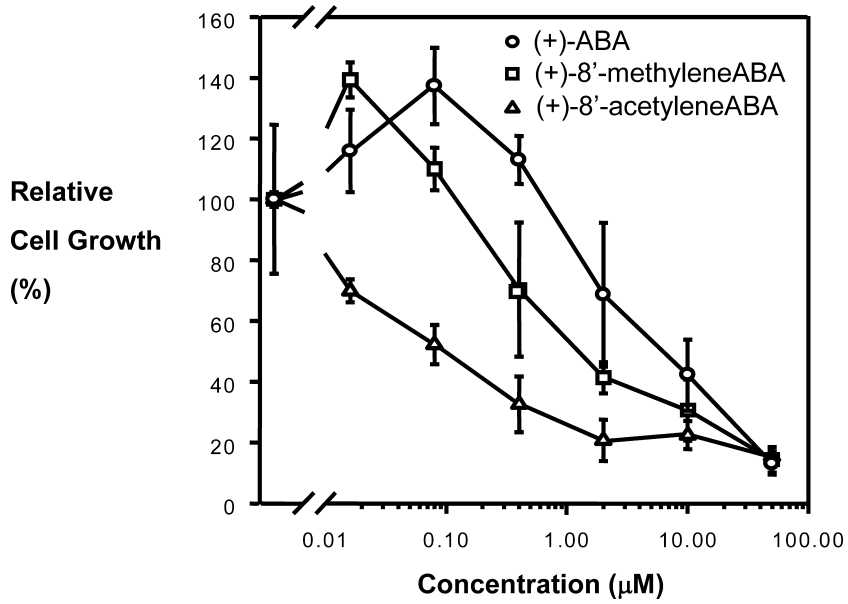

Figure 7. Effect of ABA and analogs **27** and **28** on maize cellular growth. The effect of these compounds on cell growth was calculated at each concentration by calculating the percentage increase in fresh weight after 4 days. Measurements at each concentration were performed in triplicate and the average values (± SD) were normalized to a control (untreated) value of 100%.

fused to the ring of ABA (Nyangulu and others 2005). The basic bicyclic molecule **26** has activity comparable to ABA in germination, growth inhibition, and gene expression assays (Nyangulu and others in preparation). The carboxyl, ketone, and chiral center of the ABA molecule are maintained intact in the tethered probe. The probe was validated and bound a monoclonal antibody raised against *S*-ABA, and it also bound an ABA 8′-hydroxlase enzyme expressed in yeast. Studies are now underway to employ the probe to identify ABA binding proteins in plant tissue extracts in *Arabidopsis*.

ABA Analogs—Persistent ABAs with Even a Brief Exposure

Use of *S*-ABA in practical plant-growth regulation applications and in functional tests can be hindered by rapid turnover of the hormone by plant enzymes. In practical field tests, effectiveness of ABA application can be diminished by rain and variable exposure time. This problem has largely been overcome with the development of analogs altered at the 8′-carbon atom of ABA, the site of oxidation by ABA 8′-hydroxylase (Abrams and others 1997; Rose and others 1997). Alteration by replacement of the 8′-methyl group by a methylene (**27**) or acetylene (**28**) group (Figure 6) afforded ABA analogs that are very close in structure to ABA but are resistant to oxidation at the 8′-carbon atom (Cutler and others 2000), and therefore are more persistent in plant cells (Abrams and others 1997). The biological activity of the acetylene ABA is as great or greater than ABA in every biochemical assay reported to date (Todoroki and others 1997; Cutler and others 2000; Sauter and others 2002). In a growth inhibition assay on corn cells, the inhibitory effects of both **27** and **28** were much stronger than that of ABA, as shown in Figure 7 (Loewen and Cutler, unpublished results). The methylene ABA is slowly oxidized to the epoxide, and the acetylene is much more slowly oxidized to the acetic acid derivative, consistent with P450 mono-oxygenase action (Abrams and others 1997; Cutler and others 2000). 8′-Acetylene ABA is a potent germination inhibitor of wheat embryos (from dormant grains) even when applied briefly (Figure 8). Treatment with 8′-acetylene ABA for 1–5 min, followed by washing and transfer to control buffer, effectively

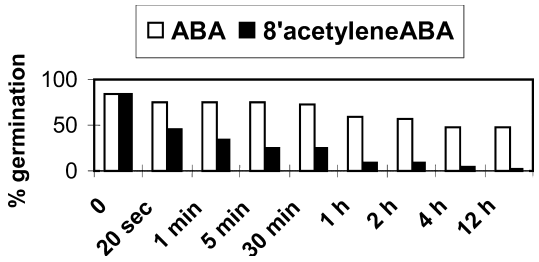

Figure 8. Effect of ABA and analog **28** on wheat embryos germination. Dormant wheat embryos were treated briefly (1–5 minutes) with 25 μm ABA or (+)-8′-acetylene ABA. The extent of germination was measured after 24 h.

suppresses germination for 24 h. The effect of this analog is markedly stronger than the natural hormone. The strong effect may be due to resistance to metabolism or possibly enhanced interaction or binding to the ABA receptor. Until the protein in which ABA binds is known, it is not possible to separate the effects of persistence and activity.

For practical applications as plant growth regulators, the racemic mixtures of analogs can be used. For some purposes, methyl esters are employed to increase uptake and transport in plants. The methyl ester of 8′-acetylene ABA (**29**, Figure 6) was effective as a root dip to slow moisture use and growth in tomato seedlings grown under greenhouse conditions (Sharma and others 2005), and in several crops, including peppers, tomatoes, and cucumbers, the effects of the racemic methyl ester analog **29** were more persistent than that of ABA (Sharma 2002). In other investigations of analog effect on prevention of salt stress damage to citrus seedlings, the methylene acid **27** was found to be the most effective treatment (Gomez-Cadenas in preparation). These analogs are practical to synthesize in quantity, and the effective dose required for treating plants is lower than that for ABA.

An ABA analog, 8′-trideuteromethyl ABA (**30**, Figure 6) was specifically designed to probe the relative biological activity of *S*-ABA versus its catabolite 8′-hydroxy ABA (Lamb and others 1996). The deuterated analog showed stronger activity than *S*-ABA in inhibiting the germination of cress seeds (*Lepidium sativum* L.) (Lamb and others 1996). This result suggests that ABA is more persistent as a result of the primary isotope effect, and that ABA itself is the active hormone. Also, **30** showed long-term activity in the rice elongation assay (Todoroki and others 1997).

Other analogs with alterations at the 7′-, 8′- and 9′-carbon atoms of the ring or alterations on the ring can also have enhanced activity and have been reviewed (Todoroki and Hirai 2002).

ABA Analogs that Probe Binding Site Structure

Optically pure ABA analogs and metabolites will be of increasing value to probe active sites of putative ABA-binding proteins as they are identified. With the few examples now in hand, patterns are emerging. It will be possible to design analogs to induce a subset of ABA-inducible genes. An early study probed the binding sites of different monoclonal antibodies raised against ABA, a model system for plant ABA binding proteins, with the finding that the individual antibodies required different parts of the ABA structure, although each had been selected for specificity to *S*-ABA (Walker-Simmons and others 1991). The first example of relating gene expression to analog structure was accomplished with a set of eight ABA compounds, in which germination in wheat embryos and induction of genes coding for *Em*, group 3 *lea* and *dhn* (*rab*) were selectively effected by analogs (Walker-Simmons and others 1992).

In a study designed to identify inhibitors of enzymes that oxidize ABA at the 8′-carbon atom, optically pure analogs were tested as inhibitors of oxidation of ABA to 8′-hydroxyABA by an extract from maize cells (Cutler and others 2000). In this case, two- and three-carbon unsaturated groups at the 8′-and 9′-carbon atom were found to be either competitive or irreversible inhibitors of the enzyme.

An ABA glucosyl transferase was found through a screen for glycosyl transferases (Lim and others 2005). An earlier report presented a glucosyl transferase that was found to accept 2–*trans*-ABA (**24**, Figure 4) preferentially over the active hormone, and thus it may not play a major role in ABA catabolism in plants (Xu and others 2002). One of the UDP-glucosyl transferases, UGT71B6, had a high selectivity for (+)-ABA and did not accept 2-*trans*-ABA or ABA catabolites as substrates. A structure/activity study with analogs altered specifically at individual sites of the ABA molecule showed that the enzyme assayed *in vitro* had no tolerance to changes at the C-8′ and C-9′ methyl groups or the C-1′ chiral center, but that it could accommodate the addition of an aromatic ring fused to the ring double bond (Priest and others 2005).

An ABA binding protein, ABA P1, was identified through an anti-idiotypic antibody route (Razem and others 2004). *In vitro* assays indicated that the protein bound *S*-ABA but did not bind *R*-ABA or the catabolites PA and DPA. Interestingly, this protein did bind the ABA precursors ABA alcohol and aldehyde, which may relate to the function of the protein *in vivo*, yet unknown.

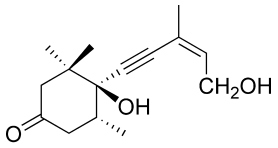

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Figure 9. An ABA analog that inhibits ABA perception.

Ueno and others (2005) probed the active site of an *Arabidopsis* ABA 8′-hydroxylase cytochrome P450 monooxygenase CYP707A3 with 45 structural analogs of ABA and concluded that substrate recognition by the enzyme strictly required the geminal methyl groups, but not the side chain methyl group (Ueno and others 2005). The side chain C-6 methyl group does appear to be required for the induction of ABA responses, and this finding may lead to the development of a specific inhibitor of the main catabolic pathway.

ABA Analogs that Inhibit ABA Perception

The activity of ABA analogs is usually compared to that of ABA, and those with less activity are discarded. One ABA analog, (–)-dihydroacetylenic abscisyl alcohol PBI-51 (**31**, Figure 9) was found to be a weak ABA analog but a competitive inhibitor of ABA perception. Its structure differs from that of S-ABA in four respects (chiral center, reduction of double bond of the ring, triple bond replacing *trans* double bond of the sidechain, and alcohol replacing the acid group). In *Brassica napus* microspore-derived embryos, this *R*-ABA-like analog inhibited ABA-induced expression of napin and oleosin genes in a dose-dependent manner (Wilen and others 1993). The antagonistic effect of this analog had been observed in enhanced germination of maize seed on treatment with the racemic mixture under low temperature conditions (Abrams and others 1993). The *S*-like form was found to be a weak ABA agonist. The analog **31** is useful for biochemical studies to alter ABA-induced gene expression, but it may not be useful for practical applications in which ABA levels in plants are not controlled.

ABA Analogs that Alter ABA Metabolism

Windsor and Zeevaart (1997) observed *R*-ABA induced increased turnover of ABA to PA in potato cell cultures. Lin and others (2005) have found that *R*-ABA increased ABA biosynthesis in *Marsilea quadrifolia* plants. The phenomenon of ABA and ABA analogs altering ABA metabolism can confound bioassay results and should be considered in biochemical investigations. Turnover of analogs can also be important in such studies.

ABA Analogs as a Negative Control

Frequently in biochemical studies, an analog of ABA is needed that can provide a rigorous negative control. The biologically inactive analog should have similar chemical properties and persistence. For experiments that are conducted entirely in the dark, photochemical product 2-*trans*-ABA (**24**) can be a good negative control. The 2-*trans*-ABA appears to be inactive, and in assays conducted in low light it is not converted to the active 2-*cis* form, depending on the plant tissue under study.

Phaseic acid is often used as a negative control in biochemical studies. In general it is thought that PA is an inactive catabolite of ABA. However, very interestingly, PA has been shown to have strong activity in a barley seed assay inhibiting GA_3-stimulated α-amylase synthesis (Todoroki and others 1995). The origin of this activity has not been studied, and could arise from opening of the oxygen bridge affording 8′-OH ABA, which has been shown to have activity in the few systems in which it has been studied.

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