Phytochemistry 96 (2013) 72–80

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Synthesis, photostability and bioactivity of 2,3-cyclopropanated abscisic acid

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ARTICLE INFO

Article history:
Received 13 December 2012
Received in revised form 24 August 2013
Available online 5 November 2013

Keywords:
Arabidopsis thaliana
Cruciferae
Abscisic acid
cis-2,3-Cyclopropanated ABA
Synthesis
Photostability
Bioactivity

ABSTRACT

The plant hormone abscisic acid (ABA) plays a central role in the regulation of plant development and adaptation to environmental stress. The isomerization of ABA to the biologically inactive 2*E*-isomer by light considerably limits its applications in agricultural fields. To overcome this shortcoming, an ABA analogue, *cis*-2,3-cyclopropanated ABA, was synthesized, and its photostability and biological activities were investigated. This compound showed high photostability under UV light exposure, which was 4-fold higher than that of (±)-ABA. *cis*-2,3-cyclopropanated ABA exhibited high ABA-like activity, including the ability to effectively inhibit seed germination, seedling growth and stomatal movements of *Arabidopsis*. In some cases, its bioactivity approaches that of (±)-ABA. *trans*-2,3-cyclopropanated abscisic acid was also prepared, an isomer that was more photostable but which showed weak ABA-like activity.

1. Introduction

Abscisic acid (ABA, **1**) is a key phytohormone that plays a central role in coordinating the physiological processes of plants, such as inhibiting or promoting growth, maintaining bud and seed dormancy and affecting flowering and sex differentiation (Gusta et al., 1988, 1994; Nambara and Marion-Poll, 2005). Regarded as an "inducer of stress resistance", ABA (**1**) can cause the plant to respond quickly to various environmental stresses, such as drought by inducing stomata closure and activating the expression of stress-resistant genes (Zeevaart and Creelman, 1988; Finkelstein et al., 2002). However, two major issues concerning the structural stability of ABA (**1**) limit its widespread application in agriculture as a plant growth regulator. One issue is oxidation by (+)-ABA 8′-hydroxylase, a cytochrome P450 mono-oxygenase that is encoded by CYP 707A, followed by isomerization of 8′-OH-ABA to inactive dihydrophaseic acid (Milborrow, 1983; Kikuzaki et al., 2004; Zaharia et al., 2004; Kushiro et al., 2004; Saito et al., 2004). Another issue is the photosensitivity of natural ABA (**1**), whose 2-*cis*-4-*trans*-3-methyl-2,4-pentadienoic acid side-chain is highly sensitive to light and isomerizes readily to the 2-*trans* geometric isomer, producing eventually a 1:1 mixture (Plancher, 1979). The 2-*trans* geometric isomer however, is biologically inactive (Milborrow, 1970; Davis et al., 1972; Walton and Sondheimer, 1972; Dashek et al., 1979; Flores and Dörffling, 1990; Balsevich et al., 1994).

At present, the problem of metabolic inactivation by the P450 enzyme has been solved successfully through the introduction of different functional groups at the 8′-position, including methoxyl (Todoroki et al., 1994), alkenyl (Abrams et al., 1997; Todoroki et al., 1997a), alkynyl groups(Todoroki et al., 1997a; Rose et al., 1997), a fluorine atom (Kim et al., 1995; Todoroki et al., 1995) or a deuterium atom (Todoroki et al., 1997b). These modifications affect the 8′-methyl transformation that leads to the inhibition of 8′-hydroxylase. Efforts to inhibit light-induced isomerization with several ABA analogues are shown in Fig. 1. To maintain the 2-*Z* configuration of the double bond in the side-chain, Ohkuma (1966) designed and synthesized compound **2** by introducing a furanone moiety in the side-chain. However, this modification destroyed the integrity of the conjugated system in the side-chain, and the terminal free carboxyl acid was fixed as a lactone, which might explain the loss of activity because both parts are required for its activity. Chen and Mactaggart (1986) tried to maintain the *cis* configuration by using a phenyl ring and synthesized compound **3**. This change resulted in a significant loss of activity with compound **3** retaining only an inhibitory effect on the germination of lettuce seeds at high concentrations. Similar modifications with a phenyl ring or a heterocyclic group in the side-chain have also been investigated by Kim et al. (1992) and Asami and Yoshida (1999),

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http://dx.doi.org/10.1016/j.phytochem.2013.09.029

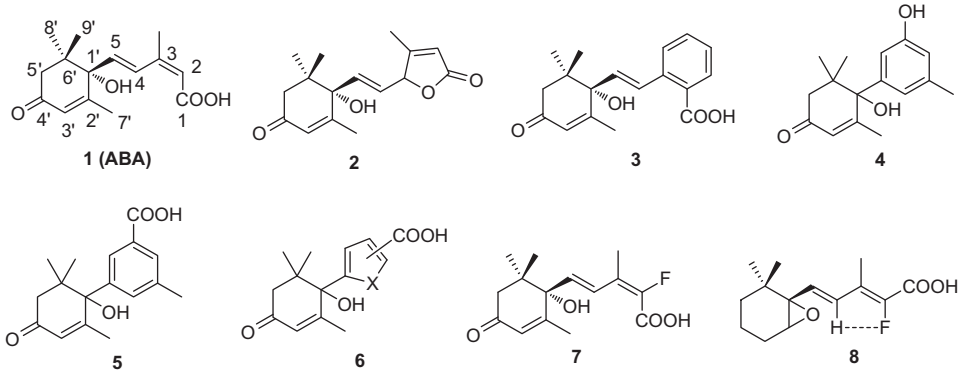


Fig. 1. Structures of ABA and some ABA analogues.

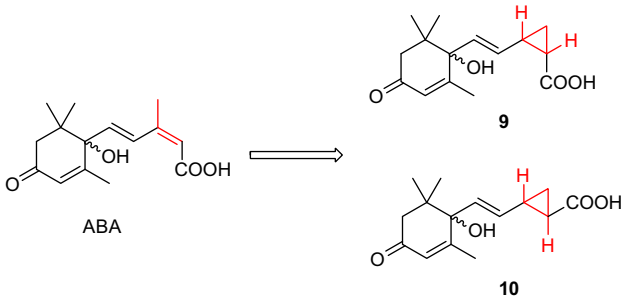
respectively. Compounds **4**–**6** were synthesized accordingly, but all showed low ABA-like activity. In an effort to increase the energy barrier for the photoisomerization of ABA (**1**), Kim et al. (1997) synthesized compound **7** by introducing a fluorine atom at the 2-position of the side-chain. Although the energy barrier was theoretically raised from 65 kcal mol^{-1} to 78.7 kcal mol^{-1} (according to quantum chemical calculation), there was no experimental proof for an improvement in the photostability of the compound. In fact, the introduction of fluorine atom might not enhance the photostability of the molecule, as Kiyota et al. (1996) demonstrated that compound **8** was much more stable than its 2-*trans* isomer due to intramolecular hydrogen bonding between hydrogen and fluorine.

While extensive efforts have been made to identify photostable ABA analogues as described above, few compounds have shown high photostability and high ABA-like activity simultaneously, most likely because of the drastic structural modifications of the side-chain. The problem of photoisomerization is still unresolved, and design and synthesis of new compounds with high photostability and good activity are still highly desirable. In this study, a novel approach to fix ABA *cis* configuration is described. The resulting ABA analogue was demonstrated to show high photostability and good ABA-like activities.

2 Results and discussion

2.1. Design and synthesis of 2,3-cyclopropanated abscisic acid

Studies of the structure–activity relationship (SAR) of ABA (**1**) indicated that the *cis* configuration of the 2-double bond, the integrity of the side-chain conjugated system and the 1-carboxyl acid are essential features for biological activity (Flores and Dörffling, 1990; Balsevich et al., 1994; Yamashita et al., 1982). The physical and chemical properties of cyclopropane are similar to those of olefins because of the increased contribution of the *p*-orbitals to the C–C bonds, whereas its steric size is larger than that of olefins (Todoroki et al., 1996). Therefore, considering the ABA (**1**) SAR results and the unique properties of cyclopropane ring, the *cis*-2,3-cyclopropanated ABA **9** (*cis*-CpABA **9**) was designed and synthesized accordingly (Scheme 1). It was anticipated that the structural changes in compound **9** would make it more stable than ABA under light exposure while maintaining much of ABA-like activity. This idea was based on the following considerations. Firstly, propylene and cyclopropane are isomers, and the essential moieties concerning biological activity were maintained in the structure of the new compound, so *cis*-cpABA **9** and ABA are bioisosteres predicted to have similar bioactivity. Secondly, the chemical properties of cyclopropane ring are similar to those of a



Scheme 1. Design strategy of 2,3-cyclopropanated ABA **9** and **10**.

double bond, substitution with a cyclopropane ring may partly maintain the integrity of the conjugated system of the side-chain. Finally, the energy barrier for *cis*–*trans* isomerization of the cyclopropane ring is generally much higher than for a double bond, so the cyclopropane ring replacement might fix the 2,3-*cis* configuration and enhance the light stability of the *cis*-isomer. Moreover, for comparison with *cis*-CpABA, the *trans* isomer, *trans*-2,3-cyclopropanated abscisic acid **10** (*trans*-CpABA **10**), was also prepared and investigated for its photostability and biological activity.

Based on the retrosynthetic analysis, the syntheses of two diastereoisomers **9** and **10** were performed mainly through the nucleophilic addition of oxoisophorone ethylene ketal **13** with the corresponding *cis*/*trans*-2-ethynyl cyclopropane carboxylate **12**, followed by selective reduction of the triple bonds to *E*-double bonds, as shown in Scheme 2. The key steps for their syntheses were to generate the *cis*-cyclopropane ring and the *trans*-cyclopropane ring, respectively. The *cis*/*trans*-cyclopropyl configurations were determined by the ^1H nuclear magnetic resonance (NMR) and nuclear overhauser effect spectroscopy (NOESY).

2.1.1. Synthesis of cis-2,3-cyclopropanated ABA 9

cis-CpABA **9** was synthesized starting from commercially available ethyl propiolate (Scheme 3). This species was converted to **14** via a Sonogashira coupling with trimethyl silyl acetylene. DIBAL-H reduction gave alcohol **15**, which could be readily converted to **17** via modified Simmons–Smith cyclopropanation method with a mild system of Sm–$HgCl_2$–CH_2I_2 (Molander, 1987, 1989), followed by deprotection to give the key intermediate. The *cis*-configuration of **17** was determined by observation of the NOE spectra of the compound **16** (Fig. 2). Both protons Ha and Hb showed NOE spectra to the same proton Hd at C-3, which indicated that the ethynyl group was *cis* to the methylol group in the cyclopropane ring. Alcohol **18** was synthesized by the nucleophilic addition of ketal **13** and alkynyl lithium, which was formed in situ by **17** and *n*-butyl