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Short communication

Synthesis and biological activity of abscisic acid esters

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ABSTRACT

16 ABA esters including 11 new compounds were prepared by two different esterification routes. All the structures of ABA esters were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. Their biological activity and hydrolysis stability were investigated. Fortunately, there were 15 and 9 compounds which displayed much better or nearly the same inhibition activity for rice seedling growth and *Arabidopsis thaliana* seed germination compared to ABA, respectively. Especially, compounds **2d** and **2g** showed better biological activities than ABA in the three tests. Moreover, we found that chemical hydrolysis ability of the esters in vitro had little relationship to their biological activity.

1. Introduction

The plant hormone (*S*)-abscisic acid (**1**, (*S*)-(+)-ABA) acting as an essential signal molecule involves in various plant growth modulation (seed germination, root elongation etc.) and abiotic stresses adaptation (drought, high salt etc.) (Wasilewska et al., 2008; Cutler et al., 2010; Kim et al., 2010). However, the widely use of the regulator suffers from inactivation by photo-isomerization (Plancher, 1979) and catabolism (Zeevaart and Creelman, 1988; Nambara and Marion, 2005). So structure modification of ABA has been one of the great interest subjects over the years. In recent years, varieties ABA derivatives with high activity have been reported. For anti-catabolism derivatives acquiring by substitution of the 8′-position of ABA using methoxyl (Todoroki et al., 1994), alkenyl, alkynyl (Todoroki et al., 1997a,b; Rose et al., 1997; Cutler et al., 2000), fluorine (Kim et al., 1995; Balko et al., 1999; Todoroki et al., 1995a,b; Hanzawa et al., 1991; Ueno et al., 2005) or deuterium (Sono et al., 1996; Lamb et al., 1996) have been provided. 2, 3-cyclopropanated ABA with excellent activity and higher photo-stability than that of natural ABA had also been reported as an anti-isomerization derivative (Liu et al., 2013).

In addition, the research of ABA carboxyl group esters is another study field of the ABA derivatives. ABA glucose esters, which could act as a storage and transport form of phytohormone, were ready to release ABA by activating β-glucosidase under stress condition (Xu et al., 2012). It has been reported that some 2-nitrobenzyl and 1-(2-nitrophenyl)ethyl esters of carboxylic of plant hormones including ABA can photolysed to the corresponding free acids in intracellular (Ward and Beale, 1995). It also has been demonstrated that compared to ABA, methyl and phenyl esters possess slightly more anti-transpiration effect than ABA itself, which might play a prolonged protection role during the crop drought (Jones and Mansfield, 1971). Moreover, the ester compounds possess better lipophilicity and permeable properties. Although the good nature of ABA esters was identified, there is no report for system bioactivity research of ABA esters.

As mentioned, ABA esters can release free ABA and corresponding hydroxyl compounds by relevant hydrolyses and intracellular photolysis. The conjugation of two or more bioactive components might exert coordination role of multiple groups (Klessig and Malamy, 1994). When we took the new compositions into account, we were curious whether hydroxyl compounds **3** (Scheme 1) possessing plant growth regulating effects might play a synergistic role with ABA after release from corresponding ABA esters.

Above all, researchers have developed hundreds of excellent ABA derivatives and analogs in laboratory, but there is no application report in agricultural field. Therefore, it is highly desirable to develop ABA derivatives with simple synthesis route and cheap and high activity for large-scale agriculture application as growth regulator. In this study, the synthesis and biological activity of 16 ABA esters were investigated. We also describe the extracellular hydrolysis of several representative esters to discuss the structure-activity relationship.

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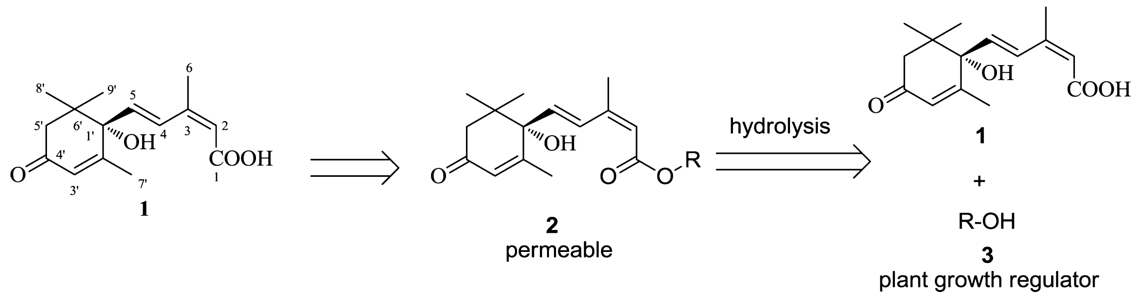


Scheme 1. Design of the ABA esters.

2. Result and discussion

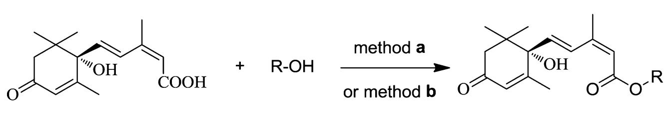
2.1. Synthesis

In view of the sensitive structure of ABA, we focused on mild esterification conditions. As shown in Scheme 2, the Steglich esterification (method **a**) (Neises and Steglich, 1978) and Mitsunobu reaction (method **b**) (Appendino et al., 2002; Mitsunobu, 1981) were applied to the synthesis of ABA ester, respectively. 12 Phenols (Table 1, **3a-3l)** and 4 alcohols **(3m-3p)** were picked as substrates to prepare the corresponding esters. In most cases, it gave an excellent yield by method **a**, except for the preparation of ABA 3-(pyridin-2-yl)propan-1-ol ester (**2o**). Instead, we got the product by method **b** with a moderate yield. All the esters were characterized by ESI-HRMS, ^{1}H and ^{13}C NMR.

2.2. Biological activity

All of 16 products were examined by three bioassays: the germination assays of *Arabidopsis thaliana* and lettuce and the seedling growth of rice. The IC_{50} values showing inhibitory effect were calculated by IBM SPSS and listed in Table 1. Besides **2a**, there are only two compounds, which were **2d** and **2g**, displayed slightly more activities than ABA for inhibiting lettuce seed germination. However, the ABA esters, whatever they were phenolic or alcohol esters, displayed high activities against rice seedling elongation. Among them, **15** compounds displayed much better or nearly the same activity compared to ABA itself, and only **2k** showed much less inhibitory effect than ABA. There were **8** compounds presented higher activity in inhibiting *A. thaliana* seed germination than ABA, and **2b** indicated the highest inhibition effect, which the IC_{50} value was 0.273 μM. It was also seen that compound **2d** showed better inhibition effect on the germination of *A. thaliana* and lettuce and the seedling growth of rice, which the IC_{50} value was 0.511 μmol/L and 1.180 μmol/L and 0.232 μmol/L, respectively. The prominent activity of some ABA esters indicated their great potential and promising prospect in field application.

We initially attributed the activity of the esters to the release rate of free ABA. First, we supposed that the ABA esters could release ABA in the external; subsequently the releasing free acid entered into the plant and then work. If the assumption was reasonable, the biology activity of the esters would be related to their chemical hydrolytic stability. Obviously, phenolic esters (compounds **2a**–**2l**) were easier hydrolysis than alcohol ester (compounds **2m–2p**), and phenolic esters with electron- withdrawing substituent on the benzene ring were more easily hydrolyzed compared to electron-donating substituent. Generally, this point was in accordance with the trend of lettuce seed germination activity. However, it could not make a reasonable explanation that ABA phenol ester (**2a**) showed more excellent activity than **2b** and **2g** and the other phenol ester bearing electron-withdrawing substituent on the benzene ring. Furthermore, there was a discrepancy in the relationship between hydrolysis speed and inhibition in *A. thaliana* germination and rice seedling elongation. Therefore, it was necessary to measure the hydrolysis rate of the esters.



Scheme 2. Synthetic routes of ABA esters.

2.3. Hydrolysis

As shown in Table 2, six representative ABA esters (**2a**, **2m**, **2h**, **2p**, **2g**, **and 2f**) were selected for hydrolysis in the condition for rice seedling growth. Seven days later, three ABA esters (**2p**, **2gand 2f**) were found obviously hydrolyzed compared to **2a**, **2m** and **2h**. Compound **2p** showed the highest hydrolysis rate, which was 96.7%, but its activity was not the best in all the bioassays, even worse than other esters in lettuce seed germination. Hence, further investigation need to be done to explore the action mechanism of the ABA esters.

As a result: (1) although some ABA esters would hydrolyze and release free ABA in environment, the relationship between hydrolysis rate and bioactivity was unclear; (2) the different activities of ABA esters might be relevant to the differences of the lipophilicity and the rate of intracellular enzyme-catalyzed hydrolysis. Based on our experiments, it was found that the bioactivity of some ABA esters were superior to ABA. We speculated the reasons as followings: the compounds were more permeable than ABA; meanwhile, the hydrolysis products of some ABA esters, which were plant growth regulatory compound and ABA, might exert some synergistic effect. It has been reported that salicylic acid is frequently used with ABA simultaneously, yielding some synergistic effect. In our experiments, compounds 2**b**, 2**c** and 2**e** contained halo-substituted phenols which are active units of some phenoxy herbicide widespread used in agriculture (Jones,1946; Muir and Hansch, 1951). It has widely known that some substituent phenols show excellent activities in many physiological processes. Nitrophenol sodium or potassium could improve germination rate and prevent flower falling and fruit dropping (Buu and Huang, 1980). Decanol could control the growth of tobacco axillary bud (Steffens and Cathey, 1969; Doss et al., 2000). *O*-phenylphenol is often used in fruit preservatives (Eckert, 1978). Pyridinepropanol inhibit reproductive stage in the vegetative growth of crops, seed rate and disease prevention. 4-Pyridinepropanol could inhibit vegetative growth and improve seed setting rate and keep plant from disease and resistance lodging in reproductive stage (Yang et al., 1986). If synergistic effects of ABA and hydroxyl compounds exist widely, it might have great meaningful for the applications of ABA esters in agricultural field.