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Synthesis of Abscisic Acid Analogs and Their Biological Activity on Growth of Rice Seedling

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합성 ABA 유도체의 벼 유묘 생장저해 작용

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Abstract : This research aims at developing a new plant growth inhibitors related to abscisic acid by means of esterification of (*S*)-(+)-ABA with p-hydroxy methyl cinnamate and umbelliferone, and testing its biological activity on growth of rice seedlings.

The over-all yield of ABA-methyl cinnamate(AC) and ABA-umbelliferone(AC) ester compounds were 83% and 78%, respectively. The growth inhibition activity of these synthetic compounds were shown about 3 to 10 times(AC) and 10 to 30 times (AU) higher than (*S*)-(+)-ABA.

Key words : (*S*)-(+)-abscisic acid, ABA-methyl cinnamate ester, ABA-umbelliferone ester, growth inhibition activity, rice seedling.

Introduction

Regulation of the growth and development of plants is dependent upon the kinds and amounts of the various hormones. Abscisic acid(ABA) has effects opposite to those of the growth promoting hormones, auxins, gibberellins, and cytokinins. Like all other plant hormones, ABA is of widespread occurrence in the plant kingdom (mono- and dicotyledons and a fern) and its presence has been detected in a variety of plant organs, including leaves, buds, fruits, seed and tubers. ABA is sesquiterpenoid compound which is related, by its biogenesis, to the monoterpenes, diterpenes, carotenoids, and triterpenes. Available evidence indicates that the naturally occurring ABA is a single enantiomorph, specifically the dextrorotatory compound, (*S*)-ABA [or (+)-ABA]. (*R*)-ABA [or (−)-ABA], which accounts for about 50% of the racemic mixtures of ABA which are made synthetically, has different biological activity on living plants to (*S*)-(+)-ABA. Naturally occurring (*S*)-(+)-ABA has also been synthesized chemically[1,2], as well as having been produced by microbial fermentation[3], and by culture broth[4]. Several substances related to ABA, *i.e.* 2-*trans*-ABA, phaseic acid, 2-*trans*-phaseic acid, 4′-dihydrophaseic acid and theaspirone, have also been found to occur in plant tissues[5]. Likewise, a number of synthetic analogs have been synthesized[5~9].

The structural features of ABA and related molecules which are important for biological activity are that : (1) carboxyl group of the C-1 position, (2) the cyclohexane ring must contain a double bond in the C-2′ and ketone group of the C-4′ position, and (3) the configuration of the C-2 double bond must be *cis*.

ABA which has specific physiological activities such as plant growth inhibition or regulation of dormancy induction tends to be easily isomerized and decomposed[10,11] to the much less active compounds. And the high cost (203$/mg) of (*S*)-(+)-ABA due to the difficulty in chemical synthesis attributed to its having stereogenic center prevents ABA from being applied to practical agricultural uses.

In the course of researches for new plant growth regulators among synthetic analogs of (*S*)-(+)-ABA, I have found that ABA-methyl cinnamate ester and ABA-umbelliferone ester possess marked biological activity. This report describes the growth inhibitory activities of these compounds on growth of rice seedlings.

Materials and Methods

Chemicals and Instruments

^{1}H-NMR spectra were recorded on a JNM-FX-200 spectrometer with tetramethylsilane as an internal standard and UV spectra were obtained with a Jasco Ubset-50 spectrophotometer. HPLC analyses were performed on a Shimadzu LC-6A with Develosil-ODS-5 column and melting points were measured with a micro-melting point from Yanagimoto Co. TLC analyses were run on Kiesel gel $60F_{254}$.

(*S*)-(+)-2-*cis*,4-*trans*-ABA used in the synthesis of ABA analogs prepared from culture medium of *B. cinerea* HP-D_8[11]. (±)-ABA, N-methyl-N-nitroso-p-toluenesulfonamide and N,N'-dicyclohexyl-carbadiimide(DCC) were purchased from Sigma Co., and 7-hydroxy coumarin(umbelliferone), p-hydroxy cinnamic acid and 4-(N,N-dimethylamino) pyridine(4-DMAP) from Junsei chemical Co. The anhydrous dichloromethane was stored over molecular sieves 4A before it was used.

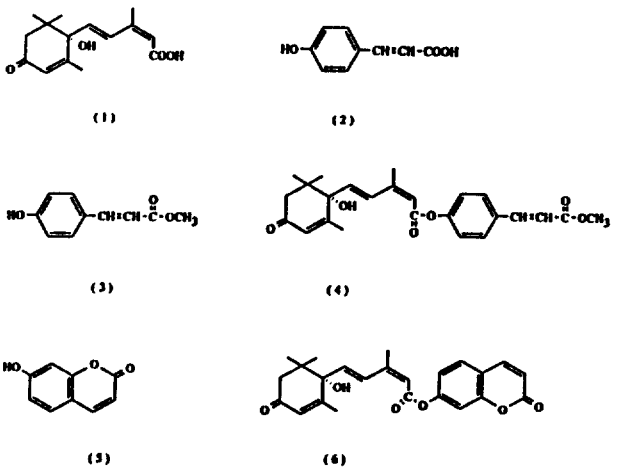
Synthesis of ABA analogs

p-Hydroxy methyl cinnamate (3) ; obtained by methylation of *p*-hydroxy cinnamic acid (2) with diazomethane.

ABA-methyl cinnamate ester (*AC*, 4) ; A mixture of 1.0g(3.8mmole) of (*S*)-(+)-ABA (1), 1.17g(5.7 mmole) of DCC, 0.2g(1.7mmole) of 4-DMAP and 1,000ml of anhydrous dichloromethane is placed in a 1,000ml flask equipped with a stirrer. The reaction mixture is stirred at 25℃ for 16h in dark. 1.09g(6.1 mmole) of p-hydroxy methyl cinnamate is then added, and the mixture is stirred for 16h. The contents of the flask are evaporated and dissolved with ethyl acetate, and washed with saturated $NaHCO_3$, H_2O and sodium chloride solution, and dried over anhydrous sodium sulfate. The ethyl acetate is removed by evaporation and the slightly yellow residue is purified by TLC on silica gel(Ben : EtOAc=1 : 1 containing 3% acetic acid, R_f=0.67). The yield of AC (4) is ca 1.33g(ca 83%), m.p. 166~168°.

ABA-umbelliferone ester (*AU*, 6) ; 0.867g(4.2 mmole) of DCC in 500ml of anhydrous dichloromethane was added to a stirred mixture of 1.0g(3.8 mmole) of (*S*)-(+)-ABA, 0.745g(4.6mmole) of umbelliferone (5) and 0.73g(0.6 mmole) of 4-DMAP in 500ml of anhydrous dichloromethane. After stirring for 12h at 25℃ in dark, it was concentrated and dissolved with ethyl acetate, and washed with saturated $NaHCO_3$, H_2O and sodium chloride solution, and dried over anhydrous sodium sulfate. The ethyl acetate is removed by evaporation and the residue is purified by TLC on silica gel (EtOAc : Hex=1 : 1, R_f=0.33). The yield of AU (6) is ca 1.2g(ca 78%), m.p. 74~76°.

cis and *trans* type of *ABA-methyl cinnamate ester* had 12.51min and 10.98min, and *cis* and *trans* type of *ABA-umbelliferone* ester had 7.10min and 6.30min of retention time with 60% acetonitrile containing 0.05% acetic acid (282nm, 1.0ml/min of flow rate).



Biological assays

The seeds of rice (*Oryza sativa* L., cv. Whayoungbyeo) was sterilized by soaking in 5% NaOCl solution twice for 5min followed by rinsing with water. The sterilized seeds were germinated in water for four days at 30℃ in the dark. The seedlings (ca 1.0mm of shoot length) were then placed in a glass tube containing 2.0ml of either a solution of ABA and its analogs at various concentrations or water alone, and the seedlings were allowed to grow, in the tube sealed with a sheet of polyethylene film under continuous illumination with a fluorescent lamp (4,000 lux, Toshiba FL 20SSD/18) and in the dark at 30℃ for 9 days.

The activity of growth inhibition by ABA and its analogs was expressed as percentage of shoot length of ABA-treated rice seedlings to that of non-treated ones, and divided into 4 groups ; above 20% (− −), 10 to 19% (−), 5 to 9% (+) and below 4% (++), from which I regarded (+) and (++) as having growth inhibition activity.

Results and Discussion

Synthesis of ABA analogs

Esterification of 1.0g (*S*)-(+)-ABA with 1.09g의 p-hydroxy methyl cinnamate gave 1.33g *AC* with 83 % yield. The melting point of AC (166~168℃) was nearly the same as that of natural (*S*)-(+)-ABA (162~163℃), and maximum UV absorption (285 nm) of AC was higher than that (249nm) of (*S*)-(+)-ABA.

Esterification of 1.0g (*S*)-(+)-ABA with umbelliferone gave 1.21g *AU* with 78% yield. The melting point of AU was 74 to 76℃, being much lower than that of (*S*)-(+)-ABA, and maximum UV adsorption of *AU* was 280nnm, being similar to that of *AC*.

^{1}H-NMR spectrum of these two analogs (Fig. 1)

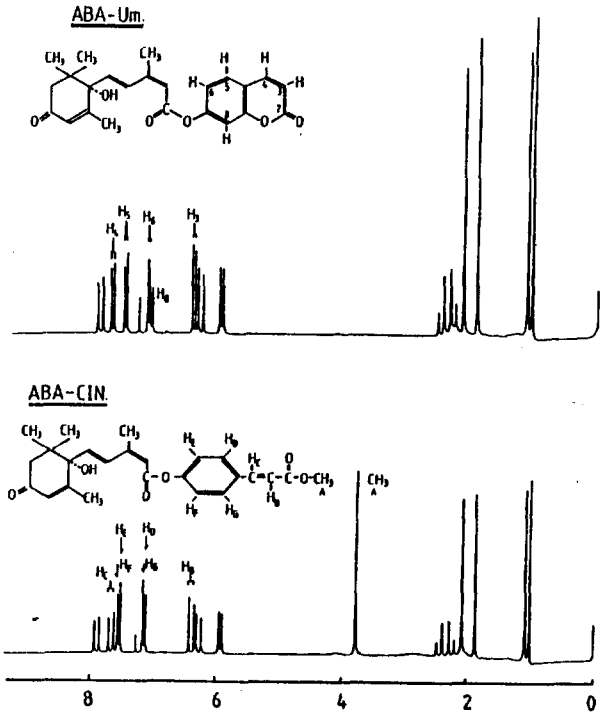


Fig. 1. ^{1}H-NMR spectra(200MHz) of AU and AC in $CDCl_3$.

δ(ppm) 1.01 (s, 3H), 1.08 (s, 3H), 1.89 (s, 3H), 2.10 (s, 3H), 2.27 (d, 1H, J=17.1), 2.46 (d, 1H, J=17.1), 3.79 (s, 3H), 5.89 (s, 1H), 5.92 (s, 1H), 6.26 (d, 1H, J=16.1), 6.37 (d, 1H, J=15.67), 7.12 (d, 2H, J=8.54), 7.53 (d, 2H, J=8.79), 7.77 (d, 1H, J=16.1), 7.89 (d, 1H, J=16.1) for AC, and 1.01 (s, 3H), 1.09 (s, 3H), 1.90 (s, 3H), 2.12 (s, 3H), 2.28 (d, 1H, J=17.09), 2.47 (d, 1H, J=17.09), 5.91 (s, 1H), 5.96 (s, 1H), 6.28 (d, 1H, J=16.12), 6.38 (d, 1H, J=9.52), 7.03 (d, 1H, J=2.2), 7.07, 7.12 (dd, 1H, J=1.95), 7.47 (d, 1H, J=8.3), 7.69 (d, 1H, J=9.52), 7.88 (d, 1H, J=16.12) for AU. Their structures 5 (AC), 7 (AU).

Biological assays

Table 1 shows the results of the growth inhibition of rice seedlings at various concentration of naturally occurring (+)-ABA and the racemic mixture in dark and in light condition, respectively. In both conditions, (±)-ABA and (+)-ABA completely inhibit growth at concentration of 40μM and 8μM, respectively. In dark (±)-ABA began to inhibit seedling growth at about 20μM whereas (+)-ABA inhibited at 6μM concentration, showing 3 times as high activity as (±)-ABA. In light (±)-ABA and (+)-ABA showed activities at 10 to 20μM and at 6μM, respectively, the activity of (+)-ABA being about 1.7 to 3 times higher than (±)-ABA.

Regardless of (*S*)-(+)-ABA, (*R*)-(−)-ABA has been shown to be highly active in various bioassays. The two enantiomers appear to be equally active inhibiting the growth of wheat embryos[12], and (*R*)-(−)-ABA strongly inhibits the growth of bean axes and barley embryos[13]. But there have been many reports suggesting that (*S*)-(+)-ABA is considerably more effective than (*R*)-(−)-enantiomer[14~17].

Table 1. Effects of (*S*)-(+)- and racemic forms of abscisic acids on the growth inhibitory activity in rice seedlings.

(M)

		4×10^{-6}	6×10^{-6}	8×10^{-6}	10^{-5}	2×10^{-5}	4×10^{-5}	6×10^{-5}	8×10^{-5}
(±)-ABA	Dark			−−	−	+	+	++	
	Light		−−	−	+	+	+	+	++
(+)-ABA	Dark	−	+	++	++				
	Light	−	+	++	++				

* −− ; above 20% of shoot length as compared with control,
− ; 10 to 19% of shoot length as compared with control
+ ; 5 to 9% of shoot length as compared with control,
++ ; below 4% of shoot length as compared with control

Table 2. The comparision of inhibitory activity between (*S*)-(+)-ABA and its analogs on rice seedlings in darkness.

(M)

	10^{-7}	2×10^{-7}	4×10^{-7}	6×10^{-7}	8×10^{-7}	10^{-6}	2×10^{-6}	4×10^{-6}	6×10^{-6}	8×10^{-6}
(+)-ABA							−−	−	+	++
AC		−−	−	+	++					
AU	−	++	++							

Table 3. The comparision of inhibitory activity between (*S*)-(+)-ABA and its analogs on rice seedlings in light.

(M)

	4×10^{-7}	6×10^{-7}	8×10^{-7}	10^{-6}	2×10^{-6}	4×10^{-6}	6×10^{-6}	8×10^{-6}
(+)-ABA					−−	−	+	++
AC			−−	−	+	++		
AU	−	++	++					

In this work, (*S*)-(+)-ABA had only one-half the inhibitory activity of racemic mixture in a growth of rice seedlings.

The growth inhibiton activity of (*S*)-ABA analogs are shown in Table 2 and 3.

AC inhibited the seedling growth at 0.6μM in dark (Table 2) and at 2μM in light (Table 3), whereas (+)-ABA did at 6μM both in dark and in light. Thus AC was 10 and 3 times more active in dark and in light than (+)-ABA, respectively. AU inhibited the seedling growth at 0.2μM in dark and at 0.6μM in light, showing 30 and 10 times as high activity in dark and in light as (+)-ABA, respectively.

Most physiological activities by about 100 ABA analogs and metabolites were reported to be lower or slightly higher than that of (*S*)-(+)-ABA. Especially among 14 ABA analogs which Tamura *et al.*[6] synthesized and tested for their activities, methyl 5-(1,2-epoxy-2,6,6-trimethyl-1-cyclohexyl)-3-methyl-*cis,trans*-2,4-pentadienoate and ethyl 5-(1,2-epoxy-2,6,6-trimethyl-1-cyclohexyl)-3-methyl-*cis,trans*-2,4-pentadienoate were found to have about 4 times as high activity as (±)-ABA and be the most excellent synthetic ABA analogs reported until now.

The functionality at C-1 plays a role in the activity of ABA. Esterification of the carboxyl group of ABA or its analogs, has been reported to increase activity[6,8,9,18], decrease activity[19], or eliminate activity[15,18]. It is likely that increased activity of the esters is due to the promotion of uptake and inhibition of metabolism, which is offset in the case of reduced activity by a slow hydrolysis to the acid that is the active species.

In this study I could synthesized two ABA analogs. *AC* having 3 times in dark and 10 times as high activity in light, and AU having 10 times in light and 30 times as high activity in dark as (*S*)-(+)-ABA. These ABA analogs have higher activities than any other ones reported until now and expected to be utilized as a plant growth regulators.

요 약

천연의 (*S*)-(+)-ABA 보다 우수한 생육저해 활성을 갖는 ABA 유도체를 개발하기 위한 연구의 일환으로 *p*-hydroxy methyl cinnamate와 umbelliferone을 (*S*)-(+)-2-*cis*,4-*trans*-ABA에 ester 결합시켜서 ABA-methyl cinnamate ester(AC)와 ABA-umbelliferone ester(AU) 화합물을 각각 83%와 78%의 높은 수율로 얻었다. 이들의 벼 유묘 생육저해 활성은 (+)-ABA에 비해서 *AC*가 3~10배, *AU*가 10~30배 정도로 훨씬 더 강한 활성을 보였다.

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