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Bioorganic & Medicinal Chemistry Letters 17 (2007) 4977–4981
Bioorganic & Medicinal Chemistry Letters

Effect of the minor ABA metabolite 7′-hydroxy-ABA on Arabidopsis ABA 8′-hydroxylase CYP707A3

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Received 14 April 2007; revised 4 June 2007; accepted 6 June 2007
Available online 10 June 2007

Abstract—To examine the effect of the minor abscisic acid (ABA) metabolite 7′-hydroxy-ABA on Arabidopsis ABA 8′-hydroxylase (CYP707A3), we developed a novel and facile, four-step synthesis of 7′-hydroxy-ABA from α-ionone. Structural analogues of 7′-hydroxy-ABA, 1′-deoxy-7′-hydroxy-ABA, and 7′-oxo-ABA were also synthesized to evaluate the role of the 7′-hydroxyl group on binding to the enzyme. The result of enzyme inhibition assay suggests that the local polarity at C-7′, neither steric bulkiness nor overall molecular hydrophilicity, would be the major reason why (+)-7′-hydroxy-ABA is not a potent inhibitor of CYP707A3.

Abscisic acid (ABA) 8′-hydroxylase (e.g., Arabidopsis CYP707A1-CYP707A4)[1,2] is a cytochrome P450 monooxygenase and a key catabolic enzyme controlling inactivation of ABA, a plant hormone involved in stress tolerance. The major ABA metabolite in most plants is 8′-hydroxy-ABA (Fig. 1), the product of ABA 8′-hydroxylase, that is easily and nonenzymatically converted into phaseic acid (PA), a low ABA activity compound. In some plants the glucose-conjugated form of ABA, ABA 1′-glucosyl ester, is the major metabolite.[3–6] The minor oxidative pathways, 7′- and 9′-hydroxylations, are also found in some plants.[7] Because Arabidopsis CYP707A3 converted ABA into neither 7′-hydroxy-ABA nor 9′-hydroxy-ABA,[1,2] these minor oxidative pathways are considered to be catalyzed by other enzymes than ABA 8′-hydroxylase.

Chemical regulation of ABA catabolism by enzyme inhibitors is a practical method to control ABA concentration. Because ABA is largely catabolized through 8′-hydroxylation by ABA 8′-hydroxylase, specific inhibitors of this enzyme are likely to be very useful tools for probing cellular and molecular events involving ABA. Recently, we proposed AHI4 as a non-azole inhibitor of ABA 8′-hydroxylase.[8] AHI4 was designed to have an axial hydroxyl group instead of the geminal methyl groups found at C-6′ of AHI1,[9] and without the enone moiety and 3-methyl (C-6) present in the ABA structure (Fig. 2). AHI4 exhibited more potent inhibitory effect on this enzyme than AHI1. This suggests that the hydroxyl group added on the ring reinforced the affinity with the active site of the enzyme. Considering that the enzyme product should egress from the active site, 8′-hydroxy-ABA can probably not bind the active site. In this case, the additional hydroxyl group will function as a substituent to decrease the affinity. The location of the additional hydroxyl group will determine whether it reinforces the affinity of the inhibitor for the active site or not. Because this knowledge is significant for designing new inhibitors of ABA 8′-hydroxylase, we are interested in whether the minor oxidative metabolites can inhibit ABA 8′-hydroxylase.

In this paper, we focused on 7′-hydroxy-ABA. Our previous work revealed that an additional methyl group at C-7′ has little effect on the enzyme inhibitory potency, whereas for C-8′ and C-9′ it largely decreased the affinity for the active site.[10] This suggests that 7′-hydroxy-ABA will not be moved out of the active site owing to its steric bulkiness. If an additional hydroxyl group

Keywords: Abscisic acid; ABA; 7′-Hydroxy-ABA; Cytochrome P450; ABA 8′-hydroxylase.
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doi:10.1016/j.bmcl.2007.06.018

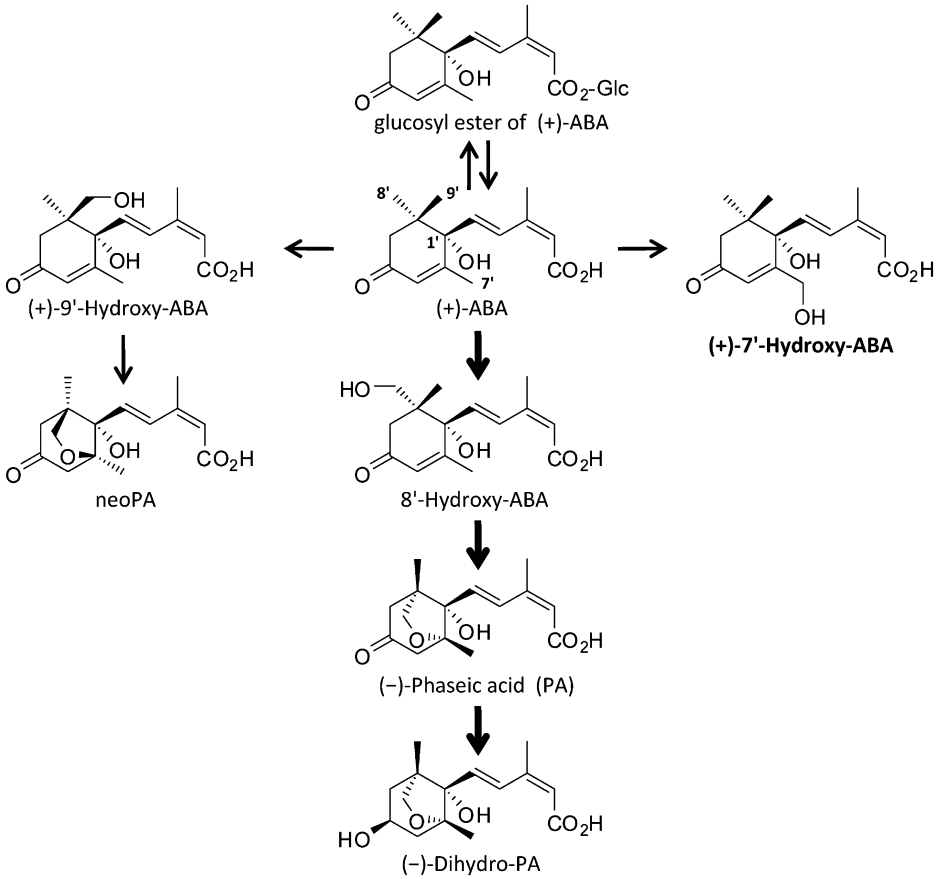


Figure 1. Catabolic pathway of (+)-ABA in plants. The key step is the 8′-hydroxylation catalyzed by ABA 8′-hydroxylase. 8′-Hydroxy-ABA is spontaneously isomerized to (−)-phaseic acid (PA), which is (probably enzymatically) reduced to give (−)-dihydro-PA. The activity of (−)-phaseic acid is one-tenth to one-hundredth of that of (+)-ABA. (−)-Dihydro-PA exhibits no ABA activity. (+)-7′-Hydroxy-ABA is more potent than (−)-PA.[19] The activity of (+)-9′-hydroxy-ABA is one-tenth of that of (+)-ABA, whereas (+)-neoPA is inactive.[20] The conjugates are inactive.

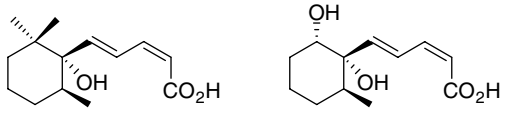
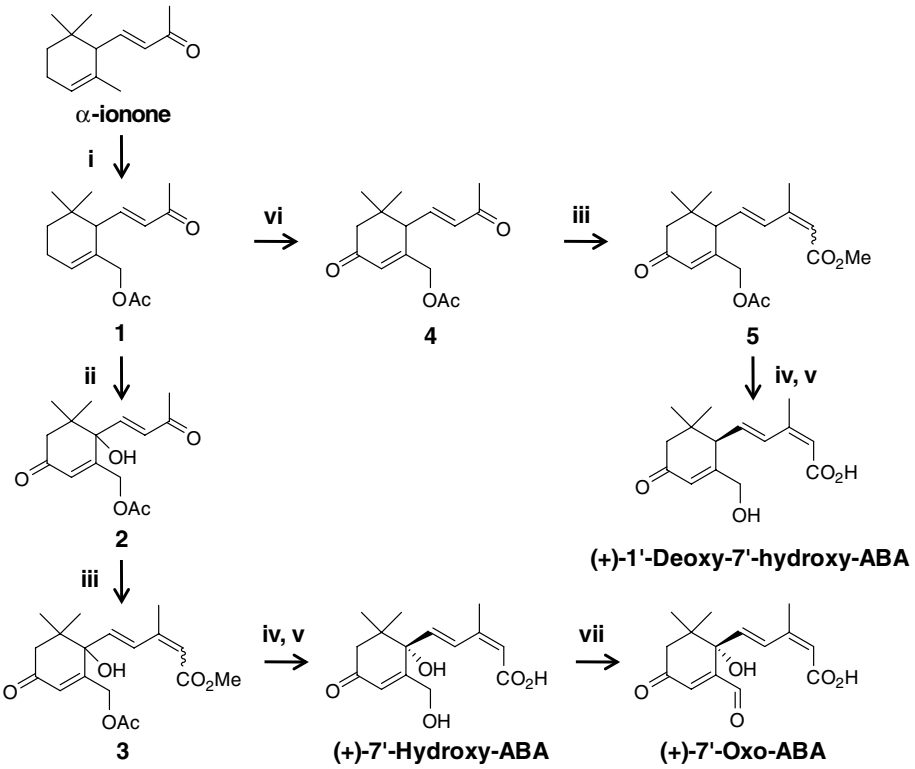


Figure 2. Chemical structures of non-azole ABA 8′-hydroxylase inhibitors.

at C-7′ reduces the affinity for the enzyme, it should depend on the higher polarity or unfavorable electrostatic interactions via the 7′-hydroxyl. Thus we prepared 1′-deoxy-7′-hydroxy-ABA and 7′-oxo-ABA in addition to 7′-hydroxy-ABA (Scheme 1). Because the hydroxyl group at C-1′ has no effect on binding to the enzyme,[10] it is considered that the absence of 1′-hydroxy and the addition of 7′-hydroxyl groups cancels each other out in respect to molecular polarity in 1′-deoxy-7′-hydroxy-ABA. This implies that we can consider the effect of the 7′-hydroxyl group itself without considering the molecular polarity. 7′-Oxo-ABA will be also less polar than 7′-hydroxy-ABA. Using this molecule as well as 7′-methyl-ABA, we discuss the effect of the local polarity at C-7′ on the interaction with the enzyme. This paper describes a novel and facile synthesis of 7′-hydroxy-ABA, 1′-deoxy-7′-hydroxy-ABA, and 7′-oxo-ABA, and their inhibitory activity against recombinant Arabidopsis ABA 8′-hydroxylase with respect to the effect of the 7′-hydroxyl group on their binding to the active site of the enzyme.

Nelson et al. reported a total synthesis of 7′-hydroxy-ABA in 1991 using a synthetic route composed of 13 steps from 1,4-cyclohexanedione monoacetal.[11] We found a novel and facile synthetic route for 7′-hydroxy-ABA, which is made of only four steps from α-ionone (Scheme 1),[12] although overall yield is low. α-Ionone was acetoxylated with iodine and silver acetate in benzene to afford **1**. Oxidation of **1** with *tert*-butyl chromate in *tert*-butanol afforded **2**. Compound **2** was converted to **3** as a 2*Z*/2*E* mixture (5:3). Hydrolysis of **3** with porcine liver esterase afforded (±)-7′-hydroxy-ABA. In this reaction, the 2*Z*-isomer was completely hydrolyzed, whereas the hydrolysis of 2*E*-isomer occurred not at C-1, but only at C-7′, to afford the methyl ester of (±)-(2*E*)-7′-hydroxy-ABA. Oxidation of **1** with pyridinium dichromate and *tert*-butyl hydroperoxide gave the deoxy compound **4**. In a similar manner for **2**, the deoxy compound **4** afforded (±)-1′-deoxy-7′-hydroxy-ABA via **5**. The racemic compounds were resolved into (+)- and (−)-isomers by HPLC employing a chiral column. Oxidation of optically pure 7′-hydroxy-ABA afforded optically pure 7′-oxo-ABA. The absolute configuration was determined based



Scheme 1. Synthesis of (+)-7′-hydroxy-ABA and its analogues. Reagents: (i) iodine, silver acetate, benzene, 19%; (ii) *tert*-butyl chromate in *tert*-butyl alcohol, acetic anhydride, 10%; (iii) bis(2,2,2-trifluoroethyl)(methoxycarbonylmethyl)phosphonate, $KN(TMS)_2$, THF, 52%; (iv) porcine liver esterase, MeOH-phosphate buffer, 89%; (v) chiral HPLC; (vi) pyridinium dichromate, *t*-BuOOH, CH_2Cl_2, 20%; (vii) MnO_2, acetone, 61%. Compounds **1**–**5** are a racemic mixture. The detailed procedure: see Supplementary data.

on the Cotton effect in the CD spectrum[13]; each (+)-isomer had the same configuration as *S*-(+)-ABA, a natural type of ABA.

Based on the calculated partition coefficient[14] (*C*log *P*) of the undissociated form and the pH-dependent distribution coefficient[15] (log *D*), (+)-7′-hydroxy-ABA is as polar as (+)-8′-hydroxy-ABA (Table 1). (+)-7′-Oxo-ABA is estimated to be less polar than (+)-7′-hydroxy-ABA, although more polar than (+)-ABA. (+)-1′-Deoxy-7′-hydroxy-ABA is calculated to be similar to (+)-ABA. In the HPLC analysis with an ODS column,[16] the retention factor (*k*) is larger in the order of 8′-hydroxy-ABA < 7′-hydroxy-ABA < 7′-oxo-ABA < ABA < 1′-deoxy-7′-hydroxy-ABA (Table 1). This almost agrees with the polarity based on the predicted partition or distribution coefficients.

Table 1. The calculated partition coefficient[14] (*C*log *P*) of the undissociated form, the pH-dependent partition coefficient[15] (log *D*), and the retention factor[16] (*k*) in the ODS HPLC analysis

Compound	*C*log *P*	log *D*			*k*
		pH 3	pH 5	pH 7	
8′-Hydroxy-ABA	0.0495	0.76	0.23	−1.60	4.86
7′-Hydroxy-ABA	0.0495	0.76	0.23	−1.60	6.34
7′-Oxo-ABA	0.4088	0.88	0.28	−1.58	9.63
ABA	0.8810	2.07	1.62	−0.17	11.4
1′-Deoxy-7′-hydroxy-ABA	1.2805	1.87	1.43	−0.37	12.8

An observed NOE between H-5 and H-5′-*proS* in the NOESY spectra (Fig. 3) of the C7′-modified analogues suggested that their favored conformation is a chair with the axial side chain. Therefore, we can discuss the effect of the local structural difference between these analogues on the affinity for the enzyme active site.

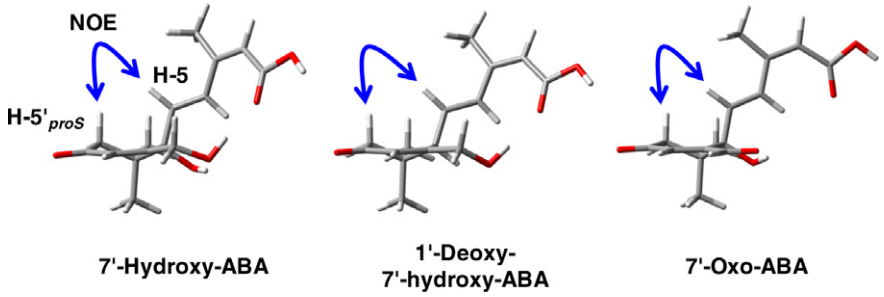


Figure 3. Favored conformations of 7′-hydroxy-ABA, 1′-deoxy-7′-hydroxy-ABA, and 7′-oxo-ABA.

Table 2. Inhibitory activity of optically pure 7′-hydroxy-ABA and its analogues against recombinant CYP707A3

Compound	Inhibition[a] (%)	
	×1	×10
(+)-7′-Hydroxy-ABA	0	0
(+)-7′-Oxo-ABA	15 ± 3	63 ± 1
(+)-1′-Deoxy-7′-hydroxy-ABA	0	24 ± 4
(+)-7′-Methyl-ABA	—[b]	83[c]
(+)-1′-Deoxy-ABA	—	73[c]

[a] Inhibition ratio in the 8′-hydroxylation for ABA (5 μM). The concentrations of compounds are 5 μM (×1) and 50 μM (×10). The inhibition ratios reported in this paper are the averages and standard deviations of three sets of experiments.
[b] Not measured.
[c] Ref. 10.

The inhibitory activity of the optically pure compounds against recombinant CYP707A3 is summarized in Table 2.[17] The activity was evaluated based on the decrease of the enzyme products, 8′-hydroxy-ABA and phaseic acid, caused by addition of a test compound at a concentration equal to or 10 times higher than the substrate (+)-ABA. (+)-7′-Hydroxy-ABA had no inhibitory effect on the enzyme reaction at the tested concentrations. (+)-1′-Deoxy-7′-hydroxy-ABA was also a very weak inhibitor, although it was a little more potent than (+)-7′-hydroxy-ABA. Only (+)-7′-oxo-ABA exhibited a significant activity, which was a little bit weaker than that of (+)-7′-methyl-ABA and (+)-1′-deoxy-ABA. All the (−)-isomers exhibited no significant inhibitory activity (data not shown).

The catalytic cycle of a cytochrome P450 enzyme is triggered as the substrate enters into the active site and displaces the axial water molecule. After the hydroxylation reaction, the hydroxylated substrate causes the entrance of bulk water molecules into the substrate-binding pocket which results in the release of the product from the enzyme.[18] This suggests that a substrate affinity for the pocket decreases as its hydrophilicity is increased. In fact, (+)-7′-hydroxy-ABA, which is the most hydrophilic compound, was the most inactive in (+)-isomers. However, (+)-1′-deoxy-7′-hydroxy-ABA was little active in spite of its more hydrophobic character compared to (+)-ABA, whereas (+)-7′-oxo-ABA was more potent than (+)-1′-deoxy-7′-hydroxy-ABA in spite of its less hydrophobic character. Steric bulkiness of the 7′-substituents is not significant in this case, because introduction of a methyl group at C-7′ has little effect on binding to the active site.[10] Likewise, the presence or absence of the oxygen at C-1′ does not affect the inhibitory potency.[10] Thus the local polarity at C-7′, neither the steric bulkiness nor overall molecular hydrophilicity, would be the major reason why (+)-7′-hydroxy-ABA is not a potent inhibitor. The potency of (+)-7′-oxo-ABA suggests that the hydrogen rather than the oxygen of the 7′-hydroxyl group causes some disadvantageous interaction with the substrate-binding cavity of the enzyme, or interferes with the approach of the compound to the cavity. The present findings will be useful for designing a specific inhibitor of CYP707A as a structural analogue of ABA, in addition to discussing the substrate binding mechanism of CYP707A.

Acknowledgment

We thank Toray Industries Inc., Tokyo, Japan, for the gift of (+)-ABA.

Supplementary data

Synthetic procedure of new compounds and their ^{1}H and ^{13}C NMR, high resolution MS, specific rotation, and circular dichroism data are given. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.018.

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12. The synthetic procedure and optical resolution of 7′-hydroxy-ABA, 1′-deoxy-7′-hydroxy-ABA, and 7′-oxo-ABA can be found in the supplementary data. 1′-Deoxy-7′-hydroxy-ABA and 7′-oxo-ABA are new ABA analogues. 1′-Deoxy-7′-hydroxy-ABA: ^{1}H NMR (500 MHz, CD_3OD): δ 1.01 (3H, s, H_3-9′), 1.06 (3H, s, H_3-8′), 2.02 (3H, s, H_3-6), 2.13 (1H, d, J = 16.8 Hz, H-5′), 2.56 (1H, d, J = 16.8 Hz, H-5′), 2.83 (1H, d, J = 9.6 Hz, H-1′), 4.14 (2H, m, H_2-7′), 5.72 (1H, s, H-2), 6.11 (1H, dd, J = 15.8 and 9.6 Hz, H-5), 6.18 (1H, s, H-3′), 7.66 (1H, d, J = 15.8 Hz, H-4); HREIMS: $[M]^+$ at m/z 264.1360 ($C_{15}H_{20}O_4$ requires 264.1362). 7′-Oxo-ABA: ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.08 (3H, s, H_3-9′), 1.14 (3H, s, H_3-8′), 2.04 (3H, d, J = 1.0 Hz, H_3-6), 2.32 (1H, d, J = 16.8 Hz, H-5′), 2.52 (1H, d, J = 16.8 Hz, H-5′), 4.36 (1H, s, HO-1′), 5.76 (1H, s, H-2), 6.25 (1H, d, J = 16.5 Hz, H-5), 6.69 (1H, s, H-3′), 7.56 (1H, d, J = 16.5 Hz, H-4), 9.75 (1H, s, H-7′); HREIMS: $[M]^+$ at m/z 278.1161 ($C_{15}H_{18}O_5$ requires 278.1154).

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16. Column, Hydrosphere C18 (S-5 μm, 12 nm, 150 × 6 mm id); mobile phase, 40% MeOH (0.1% AcOH); flow rate, 1.0 ml min^{-1}; detection, 254 nm. The retention factor (k) can be calculated according to the following equation: $k = (t_R - t_M)/t_M$, where t_M is the dead time that is taken as the first deviation of the baseline following a 5 μl injection of 1% sodium nitrite solution and t_R is the retention time.
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