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# Ring Conformational Requirement for Biological Activity of Abscisic Acid Probed by the Cyclopropane Analogues

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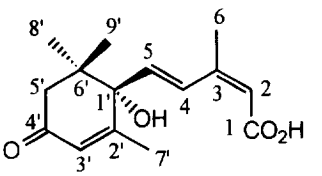
***Abstract***: For investigating ring conformational requirement for the biological activity of abscisic acid, the cyclopropane analogues **6**, **7**, **9**, and **10** were synthesized and their biological activities in four bioassays were tested. The activity of the achiral cyclohexadienone analogue **8** also was examined. Analogue **7** in which the 6'β-substituent is constrained essentially to the axial-like orientation between axial and bisectional showed no activity, while **6** and **8** with no axial-like substituent at C-6'β independent of the conformational preference exhibited the equivalent activity to abscisic acid. This result suggested that the axial substituent at the β-side of the ring is fatal to the activity. The active conformation of abscisic acid would be a conformation where C-9' is equatorial and the side chain is between pseudo-axial and bisectional, that is, close to the favored half-chair with the side chain pseudo-axial rather than the less favored half-chair with the side chain pseudo-equatorial. 

## INTRODUCTION

Abscisic acid [(1'*S*)-(+)-ABA, **1**] is the plant hormone inducing physiological responses to the environmental stresses such as drought and freezing.[1] Revealing structural requirements for the ABA activity is essential to developing the highly active analogues and photoaffinity probes for the active site on the receptor.

The ring of ABA is the 1-hydroxy-2,6,6-trimethylcyclohex-2-en-4-one with the side chain at C-1. The idealized conformations of the ring in ABA are represented using the torsion angle notation[2] in Fig. 1. In the crystal,[3] ABA adopts a slightly distorted sofa, close to the sofa $\mathbf{S}_1$, which has the non-distorted enone and pseudo-axial side chain. The preferred form in solution, revealed by NMR[4] and CD[5] analyses is the half-chair $\mathbf{HC}_1$ with the pseudo-axial side chain. The negative Cotton effect derived from the n-π* transition




**1**

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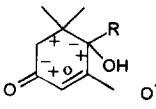
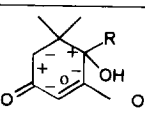
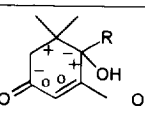
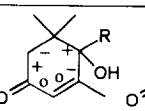
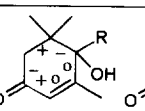
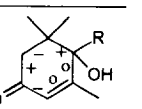
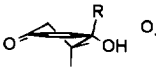
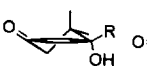
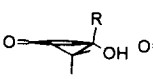
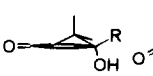
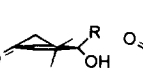
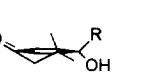
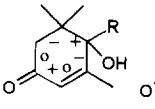
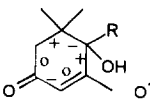
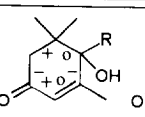
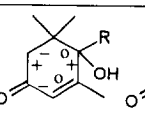
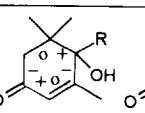
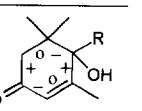
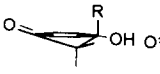
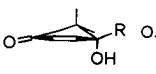
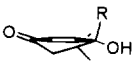
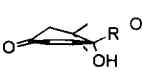
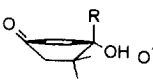
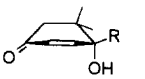
| sign of torsion angle* |  |  |  |  |  |  |
| side-view† |  |  |  |  |  |  |
| name | half-chair **$HC_1$** | half-chair **$HC_2$** | sofa **$S_1$** | sofa **$S_2$** | sofa **$S_3$** | sofa **$S_4$** |
| side-chain orientation‡ | pseudo-axial | pseudo-equatorial | pseudo-axial | pseudo-equatorial | bisectional | bisectional |
| C-9' orientation‡ | equatorial | axial | equatorial | axial | pseudo-equatorial | pseudo-axial |
| sign of torsion angle* |  |  |  |  |  |  |
| side-view† |  |  |  |  |  |  |
| name | 1,3-diplanar **$DP_1$** | 1,3-diplanar **$DP_2$** | 1,3-diplanar **$DP_3$** | 1,3-diplanar **$DP_4$** | boat **$B_1$** | boat **$B_2$** |
| side-chain orientation‡ | axial | equatorial | pseudo-axial | pseudo-equatorial | axial | equatorial |
| C-9' orientation‡ | equatorial | axial | bisectional | bisectional | pseudo-equatorial | pseudo-axial |

Fig. 1. Representation of idealized conformations of the cyclohexenone ring in ABA using the torsion angle notation and orientaions of the side chain and C-9' (R: side chain)

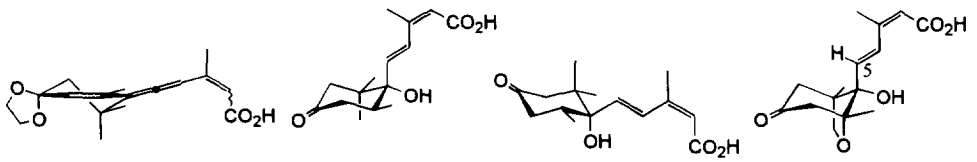
* +: clockwise torsion in the sequences taken clockwise; −: counterclockwise torsion; and 0: zero torsion.

† perspective representaion at the level of the plane formed by C-1', C-2', C-3' and C-4'; C-7' not shown.

‡ orientations to the plane including the C-2'-C-3' double bond, perpendicular to the plane formed by the side chain.

of the enone at 320 nm in the CD spectrum results from distortion of the enone,[5,6] and the value of $\Delta\varepsilon$ (-2.34)[6b] indicates that the torsion angle of C-3'-C-4' bond is about 10-20°,[6c] which almost equals the torsion angle (15°) of the most stable half-chair form of cyclohexenone.[2b] This suggests that the favored conformation in solution is not the sofa **$S_1$** but the half-chair **$HC_1$**. However, the ring of ABA is not constrained, so ABA would exist as an equilibrium mixture of some conformers, probably two, the most stable half-chair **$HC_1$** and its inverted form **$HC_2$** with the pseudo-equatorial side chain.

According to computer-aided conformational analysis,[4b] the energy difference between **$HC_1$** and **$HC_2$** is 3 kcal $mol^{-1}$, meaning that the **$HC_1$**/**$HC_2$** ratio in conformational equilibrium at 300 K is about 99.4:0.6



2 3 4 5

Fig. 2. Steric structures of the allenic analogue (**2**), dihydro analogues (**3** and **4**) and PA (**5**)

from the Gibbs equation, $\Delta G° = -RT\ln K$.[7] The energy barrier to the ring inversion between two half-chairs has not been examined. In the NMR studies of ABA by Willows and Milborrow,[4b] the spectrum at 368 K was the same as that at 300 K, so the $^1$H signals at 300 K must already be those averaging $\mathbf{HC_1}$ and $\mathbf{HC_2}$ owing to the low energy barrier to interconversion. A decrease in temperature will lead to a separation of the averaged signal into individual signals.[8] ABA has not been analyzed by low temperature NMR.

Other forms, the sofas $\mathbf{S_{1-4}}$, 1,3-diplanars $\mathbf{DP_{1-4}}$ and boats $\mathbf{B_{1-2}}$, are probably transient, short-lived conformations in the course of inverting between $\mathbf{HC_1}$ and $\mathbf{HC_2}$. Considering the low barrier to interconversion and the thermodynamic stabilization in binding to the active site on the receptor, not only half-chair forms but also these short-lived forms can be the active conformation of ABA.

The allenic analogue (**2**) has been designed and synthesized by Abrams and Milborrow[9] based on Milborrow's speculation that ABA adopts the less favored half-chair $\mathbf{HC_2}$ in binding to the uptake carrier (Fig. 2).[10] If this speculative mechanism is also necessary for binding to the receptor, then the analogue favoring the conformation with the side chain pseudo-equatorial must be more potent than or, at least as effective as ABA. However, **2** with the side chain equatorial was inactive although it showed activity after conversion to ABA.[9b] Churchill *et al.* reported that the (1'*S*,2'*S*)-2',3'-dihydro-ABA (**3**) is active, whereas (1'*S*,2'*R*)-2',3'-dihydro-ABA (**4**) is inactive.[11] The preferred conformations of **3** and **4** were chair forms with the side chain axial and equatorial, respectively, due to the steric repulsion between the 1,3-diaxial methyl groups, 7' and 9', and 7' and 8', respectively. These three examples indicate that the active conformation does not have an equatorial, but rather an axial side chain. However, phaseic acid (PA, **5**) that is a metabolite of ABA, seems to be contrary to this suggestion because PA is inactive in almost all assays[12] although its cyclohexanone ring is constrained to the chair form where the side chain is fixed in axial position owing to the bridged bicyclic system. Perhaps the side chain orientation in the active conformation of ABA is neither axial nor equatorial.

Conformational changes of ABA are almost certainly represented by the orientation of the side chain and 6'-methyl groups (C-8' and C-9') which are the bulky. Thus, investigating the orientations of these groups required for activity should be helpful in defining the active conformation. As probes, the author introduced a cyclopropane group into the ring of the ABA molecule and designed four cyclopropane analogues **6**, **7**, **9** and **10** (Fig. 3).

The physical and chemical properties of cyclopropane are similar to those of olefins, because of the increase in the *p*-orbital nature of the C-C bonds,[13] whereas its steric size is larger than that of olefins. In bicyclo[4.1.0]heptane, the cyclopropyl ring is constrained essentially in an axial-like orientation to the plane of the six-membered ring.[14] Therefore, analogues **6** and **7** where the C-5'-C-6' single bond is replaced with

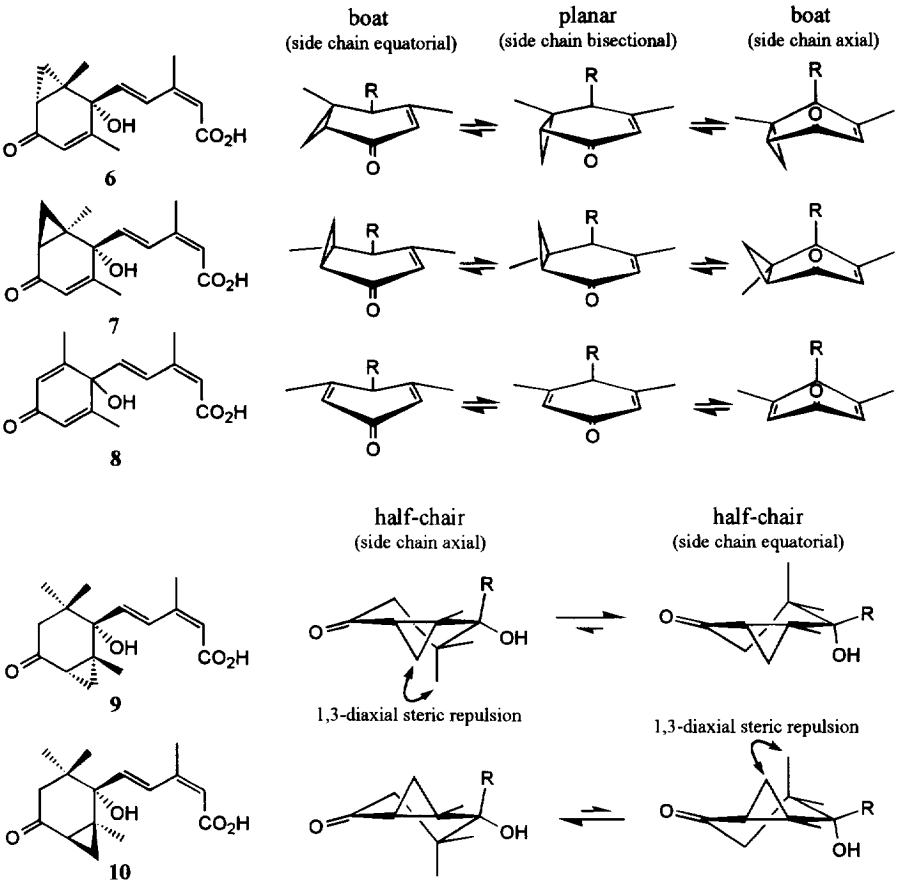



Fig. 3. Ring conformational change of **6-10** (R: side chain)

cyclopropane can possess the 6'-substituents whose orientation is constrained. Independent of the conformation of the six-membered ring, analogue **6** always possesses the axial-like 6'α- and equatorial-like 6'β-substituents, while **7** always possesses the equatorial-like 6'α- and axial-like 6'β-substituents. The six-membered ring of **6** and **7** would adopt a cyclohexadienone-like planar or boat conformation.[15] The boat-boat inversion potential of 1,4-cyclohexadiene is shallow,[15c] so **6** and **7** can convert the orientation of the side chain into that of the active form of ABA without a great loss of energy. Therefore, a comparison between the activities of **6** and **7** can afford significant understanding, not only about the steric environment around C-6' required for activity, but also the active conformation of ABA in which the orientation of the geminal methyl groups is closely related to the conformational change of the ring. Recently, the synthesis and biological activity of achiral cyclohexadienone analogue **8** has been reported to verify the pseudo-symmetric hypothesis of the ABA molecule.[16] We also synthesized **8** with the similar, planar conformation but with no cyclopropane, to evaluate the effect of the cyclopropyl ring of **6** and **7**. Replacing the 2'-double bond of ABA with cyclopropane

(**9** and **10**) would introduce 1,3-diaxial steric repulsion between the cyclopropyl ring and the 6'-methyl group in one conformer, to pull conformational equilibrium towards the other, without losing the cyclohexenone-like conformation of the six-membered ring. Compound **9** can prefer the half-chair with the pseudo-equatorial side chain similar to the disfavored conformer $\mathbf{HC_2}$ of ABA, while **10** can prefer that with the side chain pseudo-axial similar to the favored conformer $\mathbf{HC_1}$. Considering the loss of energy in changing to the active form against the 1,3-diaxial steric repulsion, the active conformation of ABA would be close to a favored conformation of the analogue that show the higher activity than the other. In this paper, we describe the synthesis and biological activity of analogues **6-10**, and discuss the conformation required for the activity.

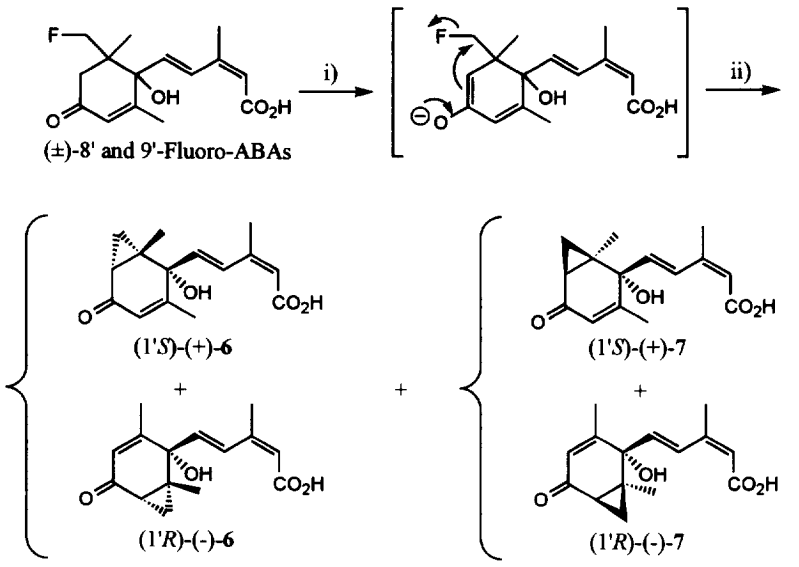
## RESULTS AND DISCUSSION

*Synthesis*

Racemic **6** and **7** were prepared by alkaline treatment of (±)-8'- and 9'-fluoro-ABAs,[17] respectively (Fig. 4). This reaction would proceed from the nucleophilic addition of the carbanion at C-5' produced under basic conditions to the electron-deficient 8'- or 9'-carbon attached to the fluorine, followed by elimination of the fluoride ion to form the cyclopropane ring. In $^1$H NMR, **6** and **7** showed signals of three coupled protons in the field higher than δ 2.0, proving the presence of a cyclopropyl ring. Racemic **6** and **7** were optically resolved by HPLC on a chiral column and (+)-**6** agreed with the compound yielded by the basic treatment of (1'*R*)-(+)-8'-fluoro-ABA, while (+)-**7** agreed with that from (1'*R*)-(+)-9'-fluoro-ABA.[17] Thus (+)-**6** and (+)-**7** were identified as (1'*S*)-(+)-5'α,8'-cyclo-ABA and (1'*S*)-(+)-5'β,9'-cyclo-ABA, respectively. Similarly, the (-)-enantiomers of **6** and **7** were identified as (1'*R*)-(-)-5'α,8'-cyclo-ABA and (1'*R*)-(-)-5'β,9'-cyclo-ABA, respectively. The absolute configurations of all the asymmetric carbons, therefore, were elucidated as (1'*S*,5'*R*,6'*S*) for (+)-**6**, (1'*S*,5'*S*,6'*R*) for (+)-**7**, (1'*R*,5'*R*,6'*S*) for (-)-**6**, and (1'*R*,5'*S*,6'*R*) for (-)-**7**.

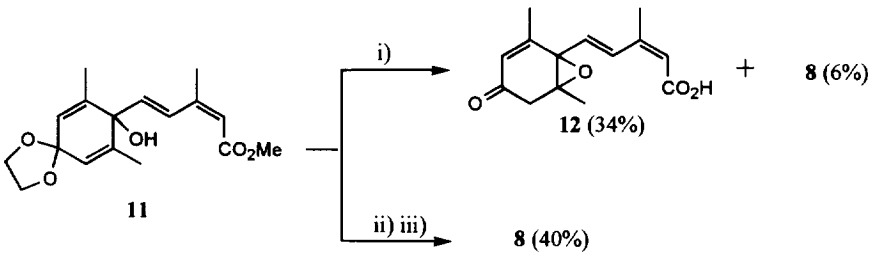
The achiral cyclohexadienone analogue **8** was synthesized according to Lei *et al*[16] except for the final step containing hydrolysis of methyl ester and deketalation at C-4' (Fig. 5). The alkaline hydrolysis of the precursor **11** and subsequent deketalation according to the reported procedure, gave the epoxy compound **12** in a 34% yield and the desired free acid **8** in an extremely low yield (6 %), which was 1/10 of the reported yield (63%). The epoxide **12** would have been yielded from the deketalated compound by Michael addition of the 1'-oxygen to the 2'- or 6'-carbon. This reaction must have been accelerated when the deketalated compound was left under basic conditions, suggesting that in adding HCl to the basic solution, the temporary, local acidic environment that allows deketalation, may occur in the basic solution. The deketalation of **11** followed by hydrolysis with esterase in phosphate buffer at pH 8 instead of in alkali, gave **8** in a 40% yield from **11**. It has been reported that **8** is very sensitive to dilute base and so unstable in solution that its biological activity could not be tested.[16] In our experiments, although **8** was unstable under strongly basic conditions, it was relatively stable in the phosphate buffer at pH 8 and in water under our assay conditions. When aqueous **8** was exposed to continuous light at 30°C for 7 days, 54% of the initial amount remained. The remaining ratio of ABA under the same conditions was 87%. Therefore, the biological activity of the free acid **8** was confirmed here for the first time. We cannot explain why our results differed from those of Lei *et al*.

Racemic **9** and **10** were synthesized from the oxoisophorone ethylene ketal **13** (Fig. 6). Compound **13** was treated with trimethyloxosulfonium iodide and sodium hydride to give the cyclopropanoid **14**.[18] The




i) NaOH ii) HPLC with a chiral column

Fig. 4. Preparation of optically active **6** and **7**




i) a) NaOH b) HCl ii) HCl iii) esterase

Fig. 5. Preparation of **8**

following synthetic route was as described by Mayer *et al.*[19] Coupling reaction of alkynyl lithium and **14** gave a mixture of two diastereomers in the ratio of about 1:10. The relative configurations of the diastereomers were determined on the basis of the NOE of the end products **9** and **10** described later; the minor diastereomer was **15a** where the cyclopropane is *trans* to the side chain, and the major diastereomer was **15b** where the cyclopropane is *cis* to the side chain. The favored conformation of **14** must be a pseudo-chair form, in which the 6-methyl group *cis* to the cyclopropane ring is in the equatorial orientation rather than the axial, which

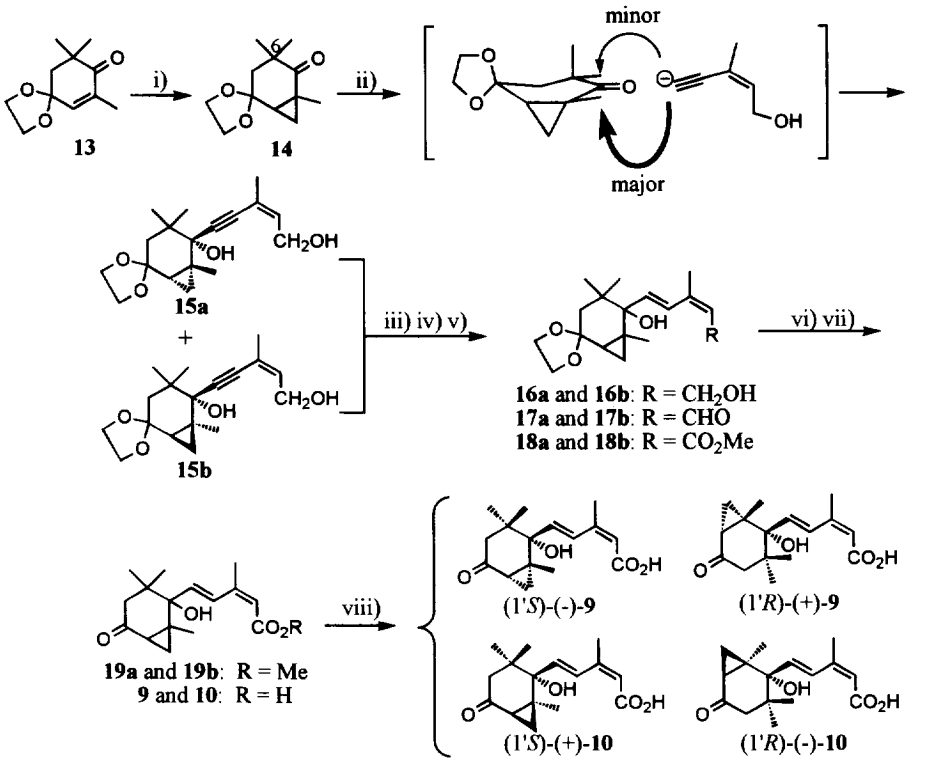



Fig. 6. Synthesis and optical resolution of **9** and **10**

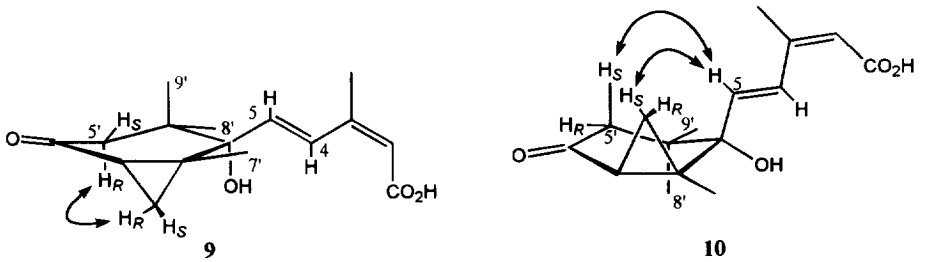



Fig. 7. Structure and favored conformation of **9** and **10** based on observed NOEs (arrows)

induces 1,3-diaxial-like steric repulsion to the cyclopropane ring, so a nucleophile, alkynyl lithium, seemed to prefer to attack the carbonyl carbon from the less hindered side, that is, the same side as the cyclopropane to mainly give **15b**. Reduction of a mixture of **15a** and **15b** gave a mixture of **16a** and **16b**, which was oxidized to a mixture of **17a** and **17b**, followed by a Corey oxidation[20] to the esters **18a** and **18b**. Acidic treatment of **18a** and **18b** separately gave **19a** and **19b**, respectively. The basic hydrolysis of **19a** and **19b** yielded the free acids **9** and **10**, respectively.

The relative configuration and favored conformation of **9** and **10** were determined by observed NOEs in the NOE difference spectra in methanol-$d_4$ (Fig. 7). The 5'*pro-R*-proton of **9** showed an NOE to the downfield 2',3'-methano proton (*pro-R*). This NOE means that **9** is (±)-2'α,3'α-dihydro-2'α,3'α-methano-ABA in which the six-membered ring favors a half-chair with the side chain pseudo-equatorial. The 5-proton of **10** showed an NOE to the downfield 2',3'-methano proton (*pro-S*) and 5'*pro-S*-proton. These NOEs mean that **10** is (±)-2'β,3'β-dihydro-2'β,3'β-methano-ABA in which the six-membered ring favors a half-chair with the side chain pseudo-axial.

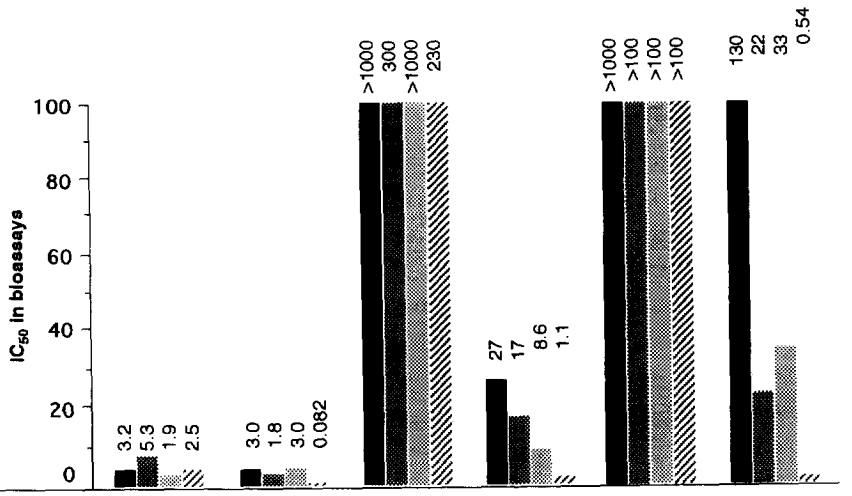
Racemic **9** and **10** were optically resolved by HPLC with a chiral column. The absolute configuration of optically active **9** and **10** was determined by the CD spectra. As described above, a cyclopropyl ring is electronically analogous to a double bond, and the cyclopropyl ketone exhibits the π-π* transition, in addition to n-π*, as an enone does.[21] This means that **9** and **10** can show split type (Davydov-split) Cotton effects at π-π* region in CD. In fact, **9** and **10** showed those, so the absolute configuration of **9** and **10** can be determined on the basis of observed Cotton effects. The results obtained in CD revealed that the absolute configuration at C-1' of (-)-**9** and (+)-**10** was equal to natural (1'*S*)-(+)-ABA, while that of (+)-**9** and (-)-**10** was equal to unnatural (1'*R*)-(-)-ABA.[6b] Therefore, the absolute configurations of all the asymmetric carbons were elucidated as (1'*S*,2'*R*,3'*S*) for (-)-**9**, (1'*S*,2'*S*,3'*R*) for (+)-**10**, (1'*R*,2'*R*,3'*S*) for (+)-**9**, and (1'*R*,2'*S*,3'*R*) for (-)-**10**.

*Biological activity*

The biological activities of analogues **6-10** were tested in the four bioassays: inhibitory effects in stomatal opening of the epidermal strips of spiderwort, lettuce seed germination, α-amylase induction by gibberellin $A_3$ in barley half-seeds and elongation of the second leaf sheath of rice seedlings. The stomata assay is a short-term assay, so the effect on this assay would mostly reflect the interaction between tested compounds and the active site of the receptor. The other assays, especially the rice assay, are affected by the rate of metabolism of tested compounds because they take a long period (2-7 days). The activities of test compounds were determined by the concentration giving half-maximal inhibition ($IC_{50}$), which, with the ring conformation and steric environment around the ring are summarized in Fig. 8.

In the stomata assay, analogues (1'*S*)-**7** and (1'*S*)-**9** were not active at all, whereas the activity of (1'*S*)-**6** was equivalent to that of (1'*S*)-ABA and those of (1'*S*)-**10** and **8** were 1/40 and 1/8 that of (1'*S*)-ABA, respectively. In assays other than the rice assay, similar results were obtained. The (1'*R*)-enantiomers were inactive in all the assays except for (1'*R*)-**6**, which was similar to (1'*R*)-ABA, 1/2 to 1/10 of (1'*S*)-ABA (data not shown).

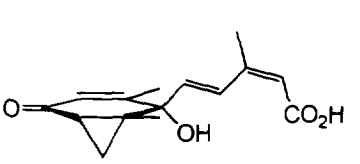
Key information for the ring conformational requirement can be drawn from a comparison of active (1'*S*)-**6**, inactive (1'*S*)-**7** and relatively active **8**. This remarkable difference in the activity among the analogues with the similar cyclohexadienone-like rings having the extremely low barrier to conformational



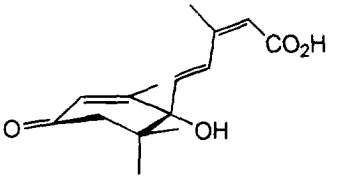

| Compound | (1'*S*)-**1** | (1'*S*)-**6** | (1'*S*)-7 | **8** | (1'*S*)-**9** | (1'*S*)-**10** |
|---|---|---|---|---|---|---|
| Ring conformation | pseudo-chair | pseudo-chair | pseudo-chair | planar | planar | planar |
| Side chain orientation | pseudo-axial | pseudo-equatorial | pseudo-axial | bisectional | bisectional | bisectional |
| axial C-2'β* | — | — | + | — | — | — |
| axial C-6'β* | — | + | — | — | — | + |

Fig. 8. The $IC_{50}$ of (1'*S*)-ABA (**1**), analogues (1'*S*)-**6**, -7,-**9**, and **10** and **8** in four bioassays: stomatal opening of the epidermal strips of spiderwort (nM), ■; lettuce seed germination (μM), ■; α-amylase induction by $GA_3$ in barley half-seeds (μM), ▒; elongation of the second leaf sheath of rice seedlings (μM), ▨, and the ring conformation and steric environments around the ring.
* The plus sign represents existence of the substituent, and the minus sign does non-existence.

change, would be attributed to the large difference in the constrained steric environments around C-6' rather than a little difference in the favored conformation. As mentioned above, analogues (1'*S*)-**6**, (1'*S*)-7 and **8** possess different orientations of the 6'-substituents independent of their conformational preference; 6'α- and 6'β-substituents of (1'*S*)-**6** are constrained to the axial-like and equatorial-like orientations, respectively, those of (1'*S*)-7 are constrained to the equatorial-like and axial-like orientations, respectively, and **8** has only one equatorial-like 6'-methyl group. Inactivity of (1'*S*)-7 should be caused by the axial-like 6'β-substituent rather than the absence of the axial-like 6'α-substituent because **8** with no such substituent at C-6' was active. This means that the axial-like 6'β-substituents prevent the activity, so the ring conformation of (1'*S*)-ABA required for activity must be a form in which C-9' is not in the axial-like orientation between axial and bisectional. The side chain in such conformations of ABA will be essentially restricted to between axial and bisectional (Fig. 1). As the absence of the 6'-methyl groups is not fatal to the activity,[22] inactivation caused by the axial-like 6'β-methyl would depend on steric hindrance against binding to the receptor, suggesting that the




(1'*R*)-**6**




(1'*R*)-ABA

receptor fits into the plane of the β-side of the ring. This mode of binding to the receptor is similar in one aspect but different in another, to that of binding to the uptake carrier as speculated by Milborrow.[10] The similarity is that the β-face of the ring is recognized, while the difference is that the ring conformation in binding cannot be the less favored half-chair $\mathbf{HC_2}$ with the side chain pseudo-equatorial as Milborrow speculated, because the half-chair $\mathbf{HC_2}$ essentially possesses the axial 6'β-methyl (C-9') that is fatal to activity.

The fatal, steric effect of the axial C-9' on activity indicates that the axial side chain can also have the same effect. If it does, PA inactivity[12] can be explained by the side chain fixed in the axial position. The lack of a 2',3'-double bond and the presence of the ether oxygen at C-2'α would have little effect on the decrease of the activity, because the dihydro analogue **3** is active,[11] and (1'*R*)-ABA and (1'*R*)-**6** which possess the axial methyl group and cyclopropyl ring which is more bulky than oxygen at the site corresponding to C-2'α of (1'*S*)-ABA, is relatively active. This also suggests that the reason why PA is ineffective, is simply the constrained conformation with the side chain axial. In binding to the active site on the receptor, ABA probably tilts the side chain to the outside of the ring, that is, to the bisectional orientation with C-9' equatorial although we cannot define the exact degree of the tilt. The active conformation of ABA may be a medium between the idealized conformations $\mathbf{HC_1}$ or $\mathbf{S_1}$ and $\mathbf{S_3}$ which can be adopted with a little increase of potential energy from the favored conformation.

The activity of the other (1'*S*)-analogues **9** and **10**, and all the (1'*R*)-analogues can be unequivocally explained by the steric effect of the axial-like substituents at the β-side of the ring. Inactive (1'*S*)-**9** possesses the axial 6'β-methyl in its favored conformation. Although (1'*S*)-**9** possesses the axial 2'α-substituent, cyclopropane ring, it would be little responsible for the inactivity because (1'*R*)-**6**, which possesses the same axial substituent at the site corresponding to C-2'α based on the pseudo-symmetric hypothesis of the ABA molecule,[10,23] was relatively active. No effect of (1'*S*)-**9** suggests that its disfavored form with no axial 6'β-methyl has much higher energy than the favored one. Analogue (1'*S*)-**10** that showed low activity possesses essentially an axial-like cyclopropyl ring at the β-side of the ring independent of its conformation. According to the pseudo-symmetric hypothesis, the inactive (1'*R*)-analogues, **7**, **9** and **10**, possess substituents corresponding to either the axial 6'β- or 2'β-substituent in (1'*S*)-ABA, while active (1'*R*)-**6** does possesses neither. The activity of the dihydro analogues **3** and **4** also can be explained by our supposed ring conformational requirement. Although the side chain in active **3** is axial, it can tilt to bisectional like ABA, because it is not fixed. Slightly tilting the axial bulky group to bisectional would necessitate only a little energy, so **3** can adopt a conformation similar to the active conformation of ABA with a little increase of energy. On the other hand, **4** which is inactive, must adopt the high energy form possessing the 1,3-diaxial methyl groups to site C-9' in the equatorial orientation.

In the rice assay, (1'*S*)-**7** and (1'*S*)-**9** were as inactive as they were in the other assays, but (1'*S*)-**6**,

(1'*S*)-**10** and **8** were more potent than (1'*S*)-ABA. Particularly, the 30-fold higher activity of (1'*S*)-**6** compared to (1'*S*)-ABA was equivalent to the activity of (1'*R*)-(+)-8',8',8'-trifluoro-ABA which is the most active analogue so far.[12b] One possible explanation for these high activities may be that these analogues are metabolized slowly; the weakened electrophilicity at C-2' for (1'*S*)-**10** and the fixed C-8' for (1'*S*)-**6** would resist the cyclization to inactive PA, and the lack of the axial 6'α-methyl for **8** would not afford a 8'-hydroxy-derivative to cyclize. It is of interest how (1'*S*)-**6** and **8** are metabolized. Stereostructures around C-6' of these analogues are almost fixed, so investigation of their sites oxidized may clarify the conformational change of ABA in binding to the active site on the 8'-hydroxylase.[24] Analogue (1'*S*)-**9** seems to resist cyclization like (1'*S*)-**10**, but the activity of (1'*S*)-**9** and its 8'-hydroxylated compound would be so low that they cannot show the increase in the activity that would be caused by the delayed metabolism since (1'*S*)-**9** has an axial substituent at C-6'β which is fatal to exhibiting the activity as described above. Another explanation may be the differences of ABA receptors of rice plant from those of the other plants.

Interaction of the α-face of the ring of ABA with the binding site has been suggested by the strict steric tolerance in the axial direction at C-2'α[23] and the role of the 1'-hydroxyl group as the hydrogen bonding donor.[17] The active site on the receptor probably recognizes both sides on the ABA ring. This information will provide important guidelines along which to develop active analogues and photoaffinity probes.

## EXPERIMENTAL

*General experimental procedures*

The $^1$H NMR spectra were recorded with TMS as an internal standard at 300 or 500 MHz using Bruker AC300 or ARX500 instrument. For clarity, the atoms of all the compounds with the carbon skeleton of ABA were numbered as in ABA in the assignment of peaks. Mass spectra were recorded at 70 eV with a Jeol JMS-DX300/DA5000 mass spectrometer. CD spectra were recorded with a Jasco J-720w spectropolarimeter. Optical rotations were measured with a Jasco DIP-1000 digital polarimeter.

*(±)-5'α,8'-Cyclo-ABA* (**6**) *and (±)-5'β,9'-Cyclo-ABA* (**7**)

To a solution of (±)-8'-fluoro-ABA[17] (50 mg, 0.169 mmol) in MeOH (5 ml) was added 1N NaOH (5 ml), and the mixture was stirred at room temperature for 3 hr and $H_2O$ (40 ml) was added. The solution was extracted with hexane (40 ml) and the aqueous layer was acidified with 1N HCl to pH 2, and extracted with EtOAc (30 ml x 3). The organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was chromatographed on silica gel (33 g) with $CH_2Cl_2$-acetone-AcOH (100:10:1 - 100:25:1) to give **6** (47.3 mg) as a white amorphous solid. In the same manner as (±)-8'-fluoro-ABA, (±)-9'-fluoro-ABA (120 mg) gave **7** (110.2 mg) as a white amorphous solid. (±)-**6**: $^1$H NMR (500 MHz, acetone-$d_6$): δ 1.08 (1H, dd, $J$ = 8.7 and 4.3 Hz, H-8'), 1.23 (3H, s, $H_3$-9'), 1.24 (1H, dd, $J$ = 4.3 and 4.3 Hz, H-8'), 1.75 (1H, ddd, $J$ = 8.7, 4.3 and 1.6 Hz, H-5'), 1.84 (1H, d, $J$ = 1.2 Hz, $H_3$-7'), 2.03 (3H, d, $J$ = 1.0 Hz, $H_3$-6), 5.52 (1H, m, H-3'), 5.76 (1H, br s, H-2), 6.02 (1H, d, $J$ = 15.8 Hz, H-5), 8.03 (1H, d, $J$ = 15.8 Hz, H-4); UV $\lambda_{max}$ (MeOH) nm (ε): 269 (17,100), 240 (15,400); IR of the methyl ester $\nu_{max}$ ($CHCl_3$) cm$^{-1}$: 3550, 3000, 1700, 1660, 1600; EIMS of the methyl ester *m/z* (rel. int.): 276 $[M]^+$ (5), 260 (7), 244 (46), 229 (35), 216 (30), 201 (44), 189 (36), 175 (56), 161 (45), 145 (39), 135 (64), 125 (100); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 276.1356 (calcd for $C_{16}H_{20}O_4$, *m/z* 276.1362). (±)-**7**: $^1$H NMR (500 MHz, $CD_3OD$): δ 1.20 (2H, m, $H_2$-9'), 1.21 (3H, s, $H_3$-8'), 1.85 (1H, m, H-5'), 1.86 (1H, d, $J$ = 1.2 Hz, $H_3$-7'), 2.06 (3H, d, $J$ = 0.9 Hz, $H_3$-

6), 5.69 (1H, m, H-3'), 5.76 (1H, br s, H-2), 6.13 (1H, d, $J$ = 16.0 Hz, H-5), 7.99 (1H, d, $J$ = 16.0 Hz, H-4); UV $\lambda_{max}$ (MeOH) nm (ε): 246 (24,500); IR of the methyl ester $\nu_{max}$ ($CHCl_3$) $cm^{-1}$: 3550, 3000, 1700, 1660, 1600; EIMS of the methyl ester *m/z* (rel. int.): 276 $[M]^+$ (1), 260 (3), 244 (14), 229 (6), 199 (7), 189 (11), 175 (7), 161 (11), 145 (6), 135 (15), 125 (100); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 276.1373 (calcd for $C_{16}H_{20}O_4$, *m/z* 276.1362).

*(2Z,4E)-5-(2',6'-Dimethyl-1'-hydroxy-4'-oxocyclohxa-2',5'-dienyl)-3-methylpent-2,4-dienoic acid* (**8**)

The methyl ester of **8** was synthesized from **11** according to the method reported by Lei *et al.*[16] To a solution of the methyl ester of **8** (40 mg, 0.15 mmol) in a mixture of MeOH (0.7 ml) and 0.1 M $KH_2PO_4$-$K_2HPO_4$ buffer (pH 8.0, 3.4 ml) was added porcine liver esterase (EC 3.1.1.1, Sigma E-3128, 0.53 ml, 1500 units). The mixture was shaken at 30°C for 16 hr, then filled up to 50 ml with $H_2O$, acidified with 1 N HCl to pH 2 and extracted with EtOAc (30 ml x 3). The organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residue was chromatographed on silica gel (4.5 g) with $CH_2Cl_2$-acetone-AcOH (70:30:1 - 60:40:1) to give **8** (26 mg, 69% yield, 40% yield from **11**) as a white amorphous powder. The spectral data of **10** agreed with those reported.

The conversion of **11** (40 mg) by the reported method[16] gave 11 mg of **12** (34% yield) and 1.8 mg of **8** (6% yield). **12**: $^1H$ NMR (500 MHz, $CDCl_3$): δ 1.41 (3H, s, $H_3$-7'), 1.99 (3H, d, $J$ = 1.1 Hz, $H_3$-8'), 2.04 (3H, d, $J$ = 1.5 Hz, $H_3$-6), 2.87 (1H, d, $J$ = 16.3 Hz, H-3'), 2.99 (1H, dd, $J$ = 16.3 and 0.6 Hz, H-3'), 5.73 (1H, br s, H-2), 6.18 (1H, d, $J$ = 16.4 Hz, H-5), 6.58 (1H, m, H-5'), 7.55 (1H, d, $J$ = 16.4 Hz, H-4); UV $\lambda_{max}$ (MeOH) nm (ε): 244 (21,300); FAB-MS (matrix, 3-nitrobenzyl alcohol) *m/z*: 249 $[M+H]^+$.

*Stability of 8*

The aqueous solutions (3 x $10^{-4}$ M) of **8** and ABA were left for 7 days under the same conditions as the rice assay. The solutions were analyzed by HPLC with an ODS column (AQ 311, 6 x 100 mm, YMC; solvent, 50% MeOH containing 0.1% AcOH; flow rate, 1.0 ml $min^{-1}$; detection, 254 nm). The recoveries of **8** and ABA were caluculated to be 54 and 87%, respectively, by being compared with the peak-heights of the standard samples.

*(±)-4,4-Ethylenedioxy-2,3-dihydro-2,3-methano-2,6,6-trimethylcyclohexan-1-one* (**14**)[19]

To a stirred solution of NaH (60% in oil, 2.9 g, 72.5 mmol) in dry DMSO (50 ml) was added trimethyloxosulfonium iodide (16 g, 72.3 mmol) at 0°C. The mixture was stirred for 15 min at 0°C. A solution of 4,4-ethylenedioxy-2,6,6-trimethylcyclohexan-1-one (**13**)[18] (11 g, 56.1 mmol) in dry DMSO (50 ml) was added with stirring and the reaction mixture was stirred at room temperature for 15 min and then at 50°C for 1 hr. After cooling and adding $H_2O$, the mixture was extracted with ether (200 ml x 3), and the organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was purified by column chromatography on silica gel (150 g) with hexane-EtOAc (9:1) to give **14** (11.8 g) as a colorless oil. $^1H$ NMR (500 MHz, $CDCl_3$): δ 1.02 (1H, dd, $J$ = 8.0 and 5.9 Hz, 2-$CH_2$-3), 1.09, 1.19 and 1.26 (each 3H, s, $H_3$-7, $H_3$-8 and $H_3$-9), 1.26 (1H, dd, $J$ = 5.9 and 5.4 Hz, 2-$CH_2$-3), 1.63 (1H, ddd, $J$ = 8.0, 5.4 and 2.1 Hz, H-3), 1.66 (1H, dd, $J$ = 14.7 and 2.1 Hz, H-5), 1.81 (1H, d, $J$ = 14.7 Hz, H-5), 4.05 (4H, m, $OCH_2CH_2O$); GC-MS *m/z* (rel. int.): 210 $[M]^+$ (11), 195 (4), 181 (5), 169 (5), 154 (18), 141 (9), 126 (100).

*(±)-(2Z)-5-(4',4'-Ethylenedioxy-2',3'-dihydro-2',3'-methano-2',6',6'-trimethylcyclohex-2'-enyl)-3-methylpent-2-en-4-yn-1-ol* (**15a** and **15b**)

To a stirred solution of *cis* 3-methylpent-2-en-4-yn-1-ol (5.8 g, 60 mmol) in dry THF (50 ml) was added dropwise *n*-BuLi (1.6 M hexane solution, 75 ml, 120 mmol) over 30 min at -78°C under nitrogen. A solution of **14** in dry THF (100 ml) was added dropwise over 30 min at room temperature and the reaction mixture was stirred for 2 hr. After cooling and adding $H_2O$, the mixture was extracted with ether (200 ml x 3), and the organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was purified by column chromatography on silica gel (180 g) with hexane-EtOAc (6:4) to give a mixture of **15a** and **15b** (4.1 g, 28.1% yield) as a colorless oil in the diastereomeric ratio of 1:10, determined by integrating the signals of the upfield 2',3'-methano proton in $^1H$ NMR spectrum. $^1H$ NMR (500 MHz, $CDCl_3$): **15a**: δ 0.60 (1H, dd, *J* = 9.7 and 5.3 Hz, 2'-$CH_2$-3'), 1.01 (1H, dd, *J* = 5.9 and 5.3 Hz, 2'-$CH_2$-3'), 1.03 (3H, s, $H_3$-8'), 1.13 (3H, s, $H_3$-9'), 1.14 (1H, ddd, J = 9.7, 5.9 and 1.6 Hz, H-3'), 1.24 (1H, dd, *J* = 14.5 and 1.6 Hz, H-5'), 1.30 (3H, s, $H_3$-7'), 1.70 (1H, d, *J* = 14.5 Hz, H-5'), 1.91 (3H, d, *J* = 1.1 Hz, $H_3$-6), 3.90-4.08 (4H, m, $OCH_2CH_2O$), 4.34 (2H, d, *J* = 6.3 Hz, $H_2$-1), 5.87 (1H, m, H-2); **15b**: δ 0.76 (1H, dd, *J* = 9.3 and 5.9 Hz, 2'-$CH_2$-3'), 0.93 (1H, dd, *J* = 5.9 and 5.5 Hz, 2'-$CH_2$-3'), 1.10 (3H, s, $H_3$-9'), 1.13 (3H, s, $H_3$-8'), 1.18 (1H, ddd, *J* = 9.3, 5.5 and 1.4 Hz, H-3'), 1.24 (3H, s, $H_3$-7'), 1.49 (1H, dd, *J* = 14.8 and 1.4 Hz, H-5'), 1.59 (1H, d, *J* = 14.8 Hz, H-5'), 1.89 (3H, d, *J* = 1.0 Hz, $H_3$-6), 2.75 (1H, s, OH), 3.90-4.08 (4H, m, $OCH_2CH_2O$), 4.31 (2H, d, *J* = 6.6 Hz, $H_2$-1), 5.86 (1H, m, H-2); EIMS *m/z* (rel. int.): 306 $[M]^+$ (2), 289 (32), 273 (29), 261 (11), 245 (19), 219 (25), 204 (35), 189 (61), 173 (55), 119 (100); HR-EIMS: $[M]^+$ at *m/z* 306.1848 (calcd for $C_{18}H_{26}O_4$, *m/z* 306.1831).

*(±)-(2Z,4E)-5-(4',4'-Ethylenedioxy-2',3'-dihydro-2',3'-methano-2',6',6'-trimethylcyclohex-2'-enyl)-3-methylpenta-2,4-dien-1-ol* (**16a** and **16b**)

To a stirred solution of a mixture of **15a** and **15b** (3.7 g, 12.1 mmol) in dry THF (50 ml) was added dropwise Red-Al® (3.4 M toluene solution, 12 ml, 40.8 mmol) in dry THF (20 ml) over 30 min at -15°C under nitrogen. The reaction mixture was stirred at room temperature for 3 hr. After cooling and adding $H_2O$, the mixture was extracted with ether (150 ml x 3), and the organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was purified by column chromatography on silica gel (40 g) with hexane-EtOAc (11:9) to give a mixture of **16a** and **16b** (2.8 g, 75.2% yield) as an oil in the diastereomeric ratio of 1:11, determined by integrating the signals of the upfield 2',3'-methano proton in $^1H$ NMR spectrum. $^1H$ NMR (500 MHz, $CDCl_3$): **16a**: δ 0.52 (1H, dd, *J* = 9.5 and 5.0 Hz, 2'-$CH_2$-3'), 0.75 (3H, s, $H_3$-8'), 1.01 (2H, m, H-3' and 2'-$CH_2$-3'), 1.06 (6H, s, $H_3$-7' and $H_3$-9'), 1.20 (1H, d, *J* = 14.3 Hz, H-5'), 1.77 (1H, d, *J* = 14.3 Hz, H-5'), 1.91 (3H, s, $H_3$-6), 3.95-4.10 (4H, m, $OCH_2CH_2O$), 4.33 (2H, m, $H_2$-1), 5.57 (1H, m, H-2), 6.13 (1H, d, *J* = 15.6 Hz, H-5), 6.79 (1H, d, *J* = 15.6 Hz, H-4); **16b**: δ 0.66 (1H, m, 2'-$CH_2$-3'), 0.96 (6H, s, $H_3$-8' and $H_3$-9'), 1.06 (3H, s, $H_3$-7'), 1.10 (2H, m, H-3' and 2'-$CH_2$-3'), 1.53 (1H, d, *J* = 14.8 Hz, H-5'), 1.65 (1H, d, *J* = 14.8 Hz, H-5'), 1.89 (3H, s, $H_3$-6), 3.41 (1H, s, OH), 3.95-4.10 (4H, m, $OCH_2CH_2O$), 4.35 (2H, m, $H_2$-1), 5.54 (1H, m, H-2), 5.79 (1H, d, *J* = 15.5 Hz, H-5), 6.72 (1H, d, *J* = 15.5 Hz, H-4); EIMS *m/z* (rel. int.): 308 $[M]^+$ (8), 290 $[M-H_2O]^+$ (6), 252 (6), 234 (20), 221 (5), 207 (5), 183 (13), 161 (14), 145 (21), 135 (15), 127 (25), 107 (23), 99 (90), 91 (25), 86 (100); HR-EIMS: $[M]^+$ at *m/z* 308.1986 (calcd for $C_{18}H_{28}O_4$, *m/z* 308.1987).

*(±)-(2Z,4E)-5-(4',4'-Ethylenedioxy-2',3'-dihydro-2',3'-methano-2',6',6'-trimethylcyclohex-2'-enyl)-3-methylpenta-2,4-dien-1-al* (**17a** and **17b**)[20]

To a solution of a mixture of **16a** and **16b** (2.7 g, 8.77 mmol) in acetone (200 ml) was added $MnO_2$ (15 g, 172 mmol) at room temperature. The suspension was stirred at room temperature for 1 hr and filtered, and the filtrate concentrated. The residual oil was purified by column chromatography on silica gel (25 g) with

hexane-EtOAc (7:3) to give a mixture of **17a** and **17b** (2.3 g, 85.7% yield) as an oil in the diastereomeric ratio of 1:11, determined by integrating the signals of the 4-H in $^1$H NMR spectrum. $^1$H NMR (500 MHz, $CDCl_3$): **17a**: δ 0.56 (1H, dd, *J* = 9.6 and 4.9 Hz, 2'-$CH_2$-3'), 0.78 (3H, s, $H_3$-8'), 1.08 (3H, s, $H_3$-9'), 1.09 (3H, s, $H_3$-7'), 1.13 (2H, m, H-3' and 2'-$CH_2$-3'), 1.23 (1H, d, *J* = 14.3 Hz, H-5'), 1.77 (1H, d, *J* = 14.3 Hz, H-5'), 2.13 (3H, d, *J* = 1.2 Hz, $H_3$-6), 3.96-4.12 (4H, m, $OCH_2CH_2O$), 5.89 (1H, d, *J* = 8.9 Hz, H-2), 6.55 (1H, d, *J* = 15.5 Hz, H-5), 7.49 (1H, d, *J* = 15.5 Hz, H-4), 10.25 (1H, d, *J* = 8.9 Hz, CHO); **17b**: δ 0.70 (1H, dd, *J* = 9.5 and 7.5 Hz, 2'-$CH_2$-3'), 0.98 (3H, s, $H_3$-9'), 1.00 (3H, s, $H_3$-8'), 1.08 (3H, s, $H_3$-7'), 1.13 (2H, m, H-3' and 2'-$CH_2$-3'), 1.55 (1H, d, *J* = 14.8 Hz, H-5'), 1.66 (1H, dd, *J* = 14.8 and 1.5 Hz, H-5'), 2.11 (3H, d, *J* = 1.0 Hz, $H_3$-6), 3.57 (1H, d, *J* = 1.4 Hz, OH), 3.96-4.12 (4H, m,$OCH_2CH_2O$), 5.86 (1H, d, *J* = 8.3 Hz, H-2), 6.20 (1H, dd, *J* = 15.4 and 1.4 Hz, H-5), 7.40 (1H, d, *J* = 15.4 Hz, H-4), 10.28 (1H, d, *J* = 8.3 Hz, CHO); EIMS *m/z* (rel. int.): 306 $[M]^+$ (2), 288 $[M-H_2O]^+$ (1), 250 (5), 235 (4), 205 (5), 177 (5), 161 (12), 127 (24), 107 (11), 99 (70), 86 (100); HR-EIMS: $[M]^+$ at *m/z* 306.1830 (calcd for $C_{18}H_{26}O_4$, *m/z* 306.1831).

*(±)-Methyl (2Z,4E)-5-(4',4'-ethylenedioxy-2',3'-dihydro-2',3'-methano-2',6',6'-trimethylcyclohex-2'-enyl)-3-methylpenta-2,4-dienoate* (**18a** and **18b**)

To a solution of a mixture of **17a** and **17b** (2.3 g, 7.5 mmol) in MeOH (100 ml) was added $MnO_2$ (10.4 g, 119 mmol), NaCN (875 mg, 17.9 mmol) and AcOH (0.44 ml, 7.6 mmol) at room temperature. The suspension was stirred at room temperature for 4.5 hr and filtered, and the filtrate was concentrated to a small volume and partitioned between ether and $H_2O$. The organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was purified by column chromatography on silica gel (35 g) with hexane-EtOAc (4:1 - 7:3) to give **18a** (0.21 g, 8.3 % yield) and **18b** (1.76 g, 69.6% yield) as colorless oils. **18a**: $^1$H NMR (500 MHz, $CDCl_3$): δ 0.52 (1H, dd, *J* = 9.5 and 5.1 Hz, 2'-$CH_2$-3'), 0.77 (3H, s, $H_3$-8'), 1.04 (2H, m, H-3' and 2'-$CH_2$-3'), 1.06 (3H, s, $H_3$-9'), 1.07 (3H, s, $H_3$-7'), 1.19 (1H, dd, *J* = 14.4 and 1.2 Hz, H-5'), 1.80 (1H, d, *J* = 14.4 Hz, H-5'), 2.05 (3H, d, *J* = 0.7 Hz, $H_3$-6), 2.29 (1H, s, OH), 3.71 (3H, s, OMe), 3.89-4.09 (4H, m, $OCH_2CH_2O$), 5.70 (1H, br s, H-2), 6.50 (1H, d, *J* = 16.0 Hz, H-5), 7.86 (1H, d, *J* = 16.0 Hz, H-4); EIMS *m/z* (rel. int.): 336 $[M]^+$ (4), 280 (5), 248 (3), 204 (9), 186 (6), 159 (12), 135 (9), 127 (15), 99 (76), 86 (100); HR-EIMS: $[M]^+$ at *m/z* 336.1947 (calcd for $C_{19}H_{28}O_5$, *m/z* 336.1937). **18b**: $^1$H NMR (500 MHz, $CDCl_3$): δ 0.68 (1H, dd, *J* = 7.8 and 5.6 Hz, 2'-$CH_2$-3'), 0.96 (3H, s, $H_3$-9'), 1.01 (3H, s, $H_3$-8'), 1.09 (3H, s, $H_3$-7'), 1.10 (2H, m, H-3' and 2'-$CH_2$-3'), 1.52 (1H, d, *J* = 14.7 Hz, H-5'), 1.67 (1H, dd, *J* = 14.7 and 0.5 Hz, H-5'), 2.03 (3H, s, $H_3$-6), 3.29 (1H, s, OH), 3.71 (3H, s, OMe), 3.94-4.11 (4H, m, $OCH_2CH_2O$), 5.68 (1H, s, H-2),6.14 (1H, d, *J* = 16.0 Hz, H-5), 7.80 (1H, d, *J* = 16.0 Hz, H-4); EIMS *m/z* (rel. int.): 336 $[M]^+$ (5), 280 (7), 248 (4), 221 (5), 204 (8), 186 (10), 159 (15), 127 (17), 107 (24), 99 (82), 86 (100); HR-EIMS: $[M]^+$ at *m/z* 336.1915 (calcd for $C_{19}H_{28}O_5$, *m/z* 336.1937).

*(±)-Methyl 2'α,3'α-dihydro-2'α,3'α-methanoabscisate* (**19a**) *and (±)-methyl 2'β,3'β-dihydro-2'β,3'β-methanoabscisate* (**19b**)

To a solution of **18a** (0.12 g, 0.357 mmol) in acetone (15 ml) was added *p*-toluenesulfonate (10 mg) at room temperature. The mixture was stirred for 4 hr and concentrated to a small volume before being added saturated aqueous $NaHCO_3$ and extracted with EtOAc (100 ml x 3). The organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was purified by column chromatography on silica gel (5 g) to give **19a** (100 mg, 95.9% yield) as a white solid. In the similar manner to **18a**, **18b** (1.85 g, 5.5 mmol) gave **19b** (1.52 g, 94.5% yield) as a colorless oil. **19a**: $^1$H NMR (500 MHz, $CDCl_3$): δ 0.86 (3H, s, $H_3$-8'), 1.00 (1H, dd, *J* = 8.9 and 3.8 Hz, 2'-$CH_2$-3'), 1.01 (3H, s, $H_3$-9'), 1.15 (3H, s, $H_3$-7'), 1.66

(1H, dd, $J$ = 14.7 and 1.0 Hz, H-5'), 1.74 (2H, m, H-3' and 2'-$CH_2$-3'), 1.74 (1H, s, OH),2.06 (3H, d, $J$ = 1.0 Hz, $H_3$-6), 2.66 (1H, d, $J$ = 14.7 Hz, H-5'), 3.72 (3H, s, OMe), 5.76 (1H, br s, H-2), 6.45 (1H, d, $J$ = 16.0 Hz, H-5), 7.94 (1H, d, $J$ = 16.0 Hz, H-4); IR $\nu_{max}$ (MeOH) $cm^{-1}$: 3600, 3500, 2950, 1690, 1635, 1600; EIMS *m/z* (rel. int.): 292 $[M]^+$ (4), 274 $[M-H_2O]^+$ (5), 260 $[M-MeOH]^+$ (7), 236 (11), 204 (31), 194 (6), 177 (40), 161 (30), 149 (38), 135 (100), 125 (36); HR-EIMS: $[M]^+$ at *m/z* 292.1685 (calcd for $C_{17}H_{24}O_4$, *m/z* 292.1675). **19b**: $^1H$ NMR (500 MHz, $CDCl_3$): δ 0.94 (3H, s, $H_3$-9'), 1.05 (3H, s, $H_3$-8'), 1.09 (1H, dd, $J$ = 9.6 and 5.8 Hz, 2'-$CH_2$-3'), 1.16 (3H, s, $H_3$-7'), 1.39 (1H, dd, $J$ = 5.8 and 4.5 Hz, 2'-$CH_2$-3'), 1.79 (1H, dd, $J$ = 9.6 and 4.5 Hz, H-3'), 1.81 (1H, s, OH), 2.04 (1H, d, $J$ = 15.8 Hz, H-5'), 2.07 (3H, s, $H_3$-6), 2.29 (1H, d, $J$ = 15.8 Hz, H-5'), 3.72 (3H, s, OMe), 5.76 (1H, br s, H-2), 6.38 (1H, d, $J$ = 16.0 Hz, H-5), 7.93 (1H, d, $J$ = 16.0 Hz, H-4); IR $\nu_{max}$ (MeOH) $cm^{-1}$: 3600, 3450, 2950, 1690, 1635, 1600; EIMS *m/z* (rel. int.): 292 $[M]^+$ (5), 274 $[M-H_2O]^+$ (5), 260 $[M-MeOH]^+$ (10), 236 (12), 204 (36), 194 (6), 177 (47), 161 (33), 149 (47), 135 (100), 125 (57); HR-EIMS: $[M]^+$ at *m/z* 292.1668 (calcd for $C_{17}H_{24}O_4$, *m/z* 292.1675).

*(±)-2'α,3'α-Dihydro-2'α,3'α-methano-ABA* (**9**) *and (±)-2'β,3'β-dihydro-2'β,3'β-methano-ABA* (**10**)

To a solution of **19a** (52 mg, 0.178 mmol) in MeOH (1 ml) was added 1N NaOH (3 ml), and the mixture was stirred at room temperature for 2.5 hr and $H_2O$ (30 ml) was added. The solution was extracted with hexane and the aqueous layer was lowered its pH to 2 with 1N HCl, and extracted with EtOAc (30 ml x 3). The organic layer was washed with $H_2O$, dried over $Na_2SO_4$, and concentrated. The residual oil was chromatographed on silica gel (5 g) with hexane-EtOAc-AcOH (28:12:1) to give **9** (46.6 mg, 94.1% yield) as a white amorphous solid. In the similar manner to **19a**, **19b** (820 mg, 2.8 mmol) gave **10** (675 mg, 86.5% yield) as a colorless oil. (±)-**9**: $^1H$ NMR (500 MHz, $CD_3OD$): δ 0.84 (3H, s, $H_3$-8'), 0.97 (3H, s, $H_3$-9'), 1.06 (1H, dd, $J$ = 9.6 and 4.8 Hz, 2'-$CH_2$-3'), 1.18 (3H, s, $H_3$-7'), 1.55 (1H, dd, $J$ = 14.5 and 1.5 Hz, H-5'*pro-S*), 1.70 (1H, ddd, $J$ = 9.6, 5.2 and 1.5 Hz, H-3'), 1.76 (1H, dd, $J$ = 5.2 and 4.8 Hz, 2'-$CH_2$-3'), 2.07 (3H, d, $J$ = 1.2 Hz, $H_3$-6), 2.69 (1H, d, $J$ = 14.5 Hz, H-5'*pro-R*), 5.74 (1H, br s, H-2), 6.52 (1H, d, $J$ = 15.9 Hz, H-5), 7.91 (1H, d, $J$ = 15.9 Hz, H-4); UV $\lambda_{max}$ (MeOH) nm (ε): 256 (19,500); EIMS *m/z* (rel. int.): 278 $[M]^+$ (3), 260 $[M-H_2O]^+$ (20), 245 (4), 222 (15), 204 (26), 194 (10), 177 (29), 161 (26), 149 (26), 135 (100), 121 (62); HR-EIMS: $[M]^+$ at *m/z* 278.1518 (calcd for $C_{16}H_{22}O_4$, *m/z* 278.1518). (±)-**10**: $^1H$ NMR (500 MHz, $CD_3OD$): δ 0.92 (3H, s, $H_3$-9'), 0.99 (3H, s, $H_3$-8'), 1.09 (1H, dd, $J$ = 9.5 and 5.7 Hz, 2'-$CH_2$-3'), 1.15 (3H, s, $H_3$-7'), 1.57 (1H, dd, $J$ = 5.7 and 4.4 Hz, 2'-$CH_2$-3'), 1.71 (1H, ddd, $J$ = 9.5, 4.4 and 1.0 Hz, H-3'), 1.85 (1H, dd, $J$ = 15.7 and 1.0 Hz, H-5'*pro-R*), 2.08 (3H, d, $J$ = 1.1 Hz, $H_3$-6), 2.52 (1H, d, $J$ = 15.7 Hz, H-5'*pro-S*), 5.73 (1H, br s, H-2), 6.53 (1H, d, $J$ = 15.9 Hz, H-5), 7.87 (1H, d, $J$ = 15.9 Hz, H-4); UV $\lambda_{max}$ (MeOH) nm (ε): 257 (18,000); EIMS *m/z* (rel. int.): 278 $[M]^+$ (2), 260 $[M-H_2O]^+$ (16), 245 (5), 222 (12), 204 (24), 194 (11), 176 (38), 161 (35), 149 (36), 135 (100), 121 (79); HR-EIMS: $[M]^+$ at *m/z* 278.1504 (calcd for $C_{16}H_{22}O_4$, *m/z* 278.1518).

*Optical resolution of* ***6, 7, 9 and 10***

Racemic **6** (10 mg) was injected into a Chiralpak AD HPLC column (250 x 4.6 mm, Daicel; solvent, 8% isopropanol in hexane containing 0.1% TFA; flow rate, 1.5 ml $min^{-1}$; detection, 254 nm). The materials at the retention times of 13.0 and 16.6 min were collected to give (+)- and (-)-**6** (5.0 and 5.0 mg) as white amorphous powders with an optical purity of 99.8 and 99.7%, respectively, measured by HPLC on the same column. (+)-**6**: $[\alpha]_D^{27}$ +527.5° ($CHCl_3$, *c* 0.333); CD $\lambda_{ext}$ (MeOH) nm (Δε): 329.6 (+3.2), 270.7 (+32.3), 218.5 (-19.2). (-)-**6**: $[\alpha]_D^{27}$ -528.4° ($CHCl_3$, *c* 0.333); CD $\lambda_{ext}$ (MeOH) nm (Δε): 329.2 (-3.5), 270.5 (-31.4), 217.2 (+19.1). Racemic **7** (15 mg) was injected into a Chiralpak AD HPLC column (solvent,

6% isopropanol in hexane containing 0.1% TFA; flow rate, 1.5 ml $min^{-1}$; detection, 254 nm). The materials at the retention times of 14.8 and 17.6 min were collected to give (+)- and (-)-**7** (6.7 and 6.4 mg) as white amorphous powders with an optical purity of 99.9 and 99.8%, respectively, measured by HPLC on the same column. (+)-**7**: $[\alpha]_D^{27}$ +200.2° ($CHCl_3$, *c* 0.213), +224.6° (MeOH, *c* 0.213); CD $\lambda_{ext}$ (MeOH) nm ($\Delta\varepsilon$): 313.2 (+5.4), 269.2 (-5.1), 239.4 (+16.5), 214.0 (-6.9). (-)-**7**: $[\alpha]_D^{27}$ -210.9° ($CHCl_3$, *c* 0.223), -207.3° (MeOH, *c* 0.223); CD $\lambda_{ext}$ (MeOH) nm ($\Delta\varepsilon$): 313.1 (-5.1), 269.0 (+4.8), 239.2 (-15.5), 213.1 (+7.3). Racemic **9** (22 mg) was injected into a Chiralcel OD HPLC column (250 x 4.6 mm, Daicel; solvent, 7% isopropanol in hexane containing 0.1% TFA; flow rate, 1.0 ml $min^{-1}$; detection, 254 nm). The materials at the retention times of 10.2 and 16.8 min were collected to give (-)- and (+)-**9** (10.1 and 10.1 mg) as white amorphous powders with an optical purity of 99.7 and 99.2%, respectively, measured by HPLC on the same column. (-)-**9**: $[\alpha]_D^{25}$ -21.4° (MeOH, *c* 0.673); CD $\lambda_{ext}$ (MeOH) nm ($\Delta\varepsilon$): 283.4 (-0.9), 255.4 (+3.3), 207.6 (-13.5). (+)-**9**: $[\alpha]_D^{25}$ +19.8° (MeOH, *c* 0.667); CD $\lambda_{ext}$ (MeOH) nm ($\Delta\varepsilon$): 284.6 (+1.1), 258.3 (-3.2), 205.6 (+14.5). Racemic **10** (14 mg) was injected into a Chiralcel OD HPLC column (solvent, 8% isopropanol in hexane containing 0.1% TFA; flow rate, 1.0 ml $min^{-1}$; detection, 254 nm). The materials at the retention times of 12.6 and 18.4 min were collected to give (-)- and (+)-**10** (6.7 and 6.6 mg) as white amorphous powders with an optical purity of 99.9 and 99.2%, respectively, measured by HPLC on the same column. (-)-**10**: $[\alpha]_D^{25}$ -68.0° (MeOH, *c* 0.447); CD $\lambda_{ext}$ (MeOH) nm ($\Delta\varepsilon$): 247.0 (-3.7), 212.7 (+3.3). (+)-**10**: $[\alpha]_D^{25}$ +67.3° (MeOH, *c* 0.440); CD $\lambda_{ext}$ (MeOH) nm ($\Delta\varepsilon$): 248.4 (+3.8), 214.0 (-2.5).

*Preparation of optically active **6** and **7** from optically active 8'- and 9'-fluoro-ABAs*[17]

To a solution of (1'*R*)-(+)-8'-fluoro-ABA (100 μg) in MeOH (20 ml) was added 1N NaOH (50 μl). The mixture was left for 1 hr at room temperature, acidified with 1N HCl to pH 2 and extracted with EtOAc (0.2 ml x 5). The organic layer was concentrated to give the bicyclic compound, which gave the same retention time as (-)-**6** under the same HPLC condition that racemic **6** was optically resolved. In the same manner as (1'*R*)-(+)-8'-fluoro-ABA, (1'*S*)-(-)-8'-fluoro-ABA gave (+)-**6**, (1'*R*)-(+)-9'-fluoro-ABA did (+)-**7** and (1'*S*)-(-)-9'-fluoroabscisic acid did (-)-**7**.

*Bioassays*

Details have been reported previously.[25] For lettuce germination assay, the number of germinated lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds was counted after incubation with the test solution at 25°C for 48 hr. For rice seedling elongation assay, the length of the second leaf sheath of rice (*Oryza sativa* L. cv. Nihonbare) seedlings was measured after incubation with the test solution in continuous light at 30°C for 7 days. For α-amylase assay, after incubating barley (*Hordeum vulgare* L. cv. Himalaya) half-seeds without embryos in the test solution at 30°C for 48 hr in the dark, the absorbance of the test solution at 660 nm was measured by the Somogyi-Nelson method. For stomata assay, the width of stomatal apertures on epidermal strips of spiderwort (*Tradescantia reflexa* Rafin) was measured after incubation with the test solution in continuous light at 25°C for 3 hr.

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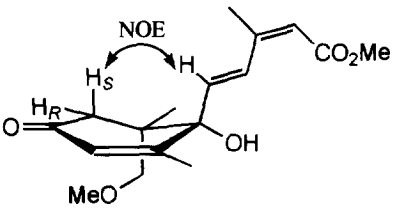
In this paper, we reported that the methyl ester of 8'-methoxy-ABA would adopt a half-chair with the side chain pseudo-equatorial in methanol-$d_4$ in the basis of the W coupling observed between the 3'-proton and the downfield 5'-proton, and no NOE between the 5- and 5'-protons. However, when we repeated the NOE experiment, an NOE between the 5-proton and the upfield 5'-proton was observed in a good reproducibility. We revised the favored ring conformation of the methyl ester of 8'-methoxy-ABA in methanol-$d_4$ : it adopts a half-chair with the side chain pseudo-axial as ABA, and the upfield 5'-proton is the *pro-S* proton, contrary to the case of ABA.

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