(2)

STUDIES ON HIGHLY POTENT ANALOGUES OF ABSCISIC ACID

YASUSHI TODOROKI

1996

Contents

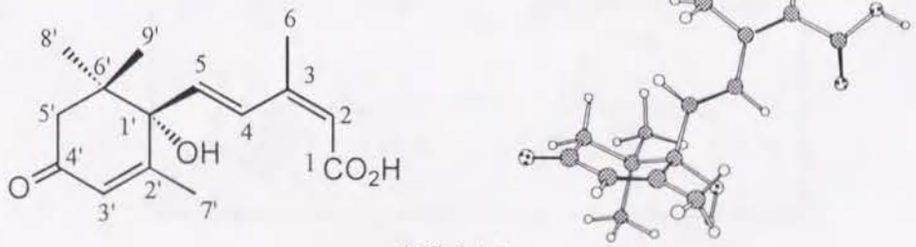
Abbreviations

ABA	Abscisic acid
ABA-GE	1-*O*-Glucosyl ester of abscisic acid
ABA-GS	1'-*O*-Glucoside of abscisic acid
Ac_2O	Acetic anhydride
AcOH	Acetic acid
BPO	Benzoyl peroxide
Bu_4NF	Tetrabutylammonium fluoride
n-BuLi	*n*-Butyl lithium
CD	Circular dichroism
DAST	Diethylaminosulphur trifluoride
DPA	Dihydrophaseic acid
epi-DPA	*epi*-Dihydrophaseic acid
EIMS	Electron impact mass spectrometry
$Et(i\text{-}Pr)_2N(HF)_3$	*N*-Ethyldiisopropylamine tris(hydrofluoride)
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
FAB-MS	Fast atom bombardment mass spectrometry
GA_3	Gibberelline A_3
GC-MS	Gas chromatography-mass spectrometry
GLC	Gas-liquid chromatography
8'-HOABA	8'-Hydroxyabscisic acid
HPLC	High performance liquid chromatography
HR-EIMS	High resolution electron impact mass spectrometry
IC_{50}	Concentration giving half-maximal inhibition
LDA	Lithium diisopropylamide
LUMO	Lowest unoccupied molecular orbital
Me_3SOI	Trimethyloxosulfonium iodide
NBS	*N*-Bromosuccinimide
NOE	Nuclear Overhauser effect
PA	Phaseic acid
PCC	Pyridinium chlorochromate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
p-TsOH	*p*-Toluenesulfonate

General Introduction

Abscisic acid [(1'*S*)-(+)-ABA (**1**), Fig. 1] is a plant hormone which was identified as a growth inhibitor, a dormancy factor and an abscission accelerator in the 1960's.[1] It has been proven ubiquitous in higher plants.[1-3] ABA is involved in the regulation of many physiological processes which are unique and cannot be substituted by the other plant hormones, ranging from the suppression of growth and germination, to the enhancement of adaptation to various stresses such as reduced transpiration by stomatal closure, induced freezing tolerance, and the activation of defense-related genes after mechanical damage to plant tissue.[1-3]

Recent topics of ABA research have included the pathway of biosynthesis, signal transduction, ABA-responsive genes, and behavior as a stress hormone.[3] There has been debate as to whether the biosynthesis of ABA proceeds via a direct sesquiterpenoid (C15) pathway or via an indirect carotenoid (C40) pathway, but investigators now tend to favor the latter.[4-6] The carotenoid pathway proceeds as follows: all-*trans*-violaxanthin, 9'-*cis*-neoxanthin, xanthoxin, ABA-aldehyde, ABA. ABA stimulates changes in the concentration of cytosolic free calcium ions in the guard cells and other tissues.[7,8] There may be a calcium-based signal transduction pathway through interaction with phosphoinositide metabolism that regulates stomatal closure and other physiological responses, as in animal cells. Several ABA-responsive genes and their gene products have been identified.[9,10] Dehydrin is expressed during the late stage of embryogenesis, and it could protect the embryo from desiccation.[11,12] Proteinase inhibitor II is involved in the defense mechanism against wounding and infection in tomato and potato plants.[13] It has been suggested that endogenous ABA induces the production of these proteins. The ABA-responsive gene ABI1 of *Arabidopsis thaliana* has been cloned by two groups, who showed that the ABI1 gene encodes a Ca^{2+}-dependent phosphatase.[14,15] The control of the phosphorylation state of cell signaling components by the phosphatase could mediate pleiotropic hormonal responses. Biophysical studies of ABA have suggested that it is the only plant hormone that can act as a stress messenger that is ideally distributed and redistributed according to pH-shifts in stressed and non-stressed plant tissues.[16] ABA can transduce environmental stresses such as desiccation, freezing and wounding into defensive responses at the molecular, cellular and whole plant levels.[3]



(1'*S*)-(+)-**1**

Fig. 1. Structural formula of (1'*S*)-(+)-ABA (**1**) showing conventional numbering system (left) and crystal form[164,165] of the molecule (right).

In contrast to the above areas, studies on the binding proteins including the ABA receptor and metabolic enzymes have not progressed as far. Few efforts have been made to identify and characterize the ABA receptor, and localization at the cellular level remains unclear (see Chapter II.1). Understanding of the metabolic enzymes associated with ABA has not made much progress since 1976 (see Chapter I.1).

The application of ABA to agricultural production has not been studied in detail. The role of ABA as a stress hormone would permit a great contribution to agriculture if its application to plants in the fields can effect the same responses as endogenous ABA. However, the commercial applications of ABA remain minimal.[17] The most critical obstruction to agricultural application is the short-lived activity of applied ABA.[17] This is a serious drawback to its use as a practical plant growth regulator.

The short-life of ABA is attributed to photoisomerization in the environment and its rapid metabolic inactivation after incorporation into plants.[17] One approach to enhance the effectiveness of applied ABA would be to supply it in the form of an analogue with an extended half-life through reduced photoisomerization and metabolism, or extremely high affinity for the active site on the receptor.

The author has designed and synthesized ten highly potent analogues of ABA from the following two standpoints: resistance to metabolic inactivation and increased affinity for the receptor. Figure 2 summarizes the synthesized analogues with classification based on the design concept. The one hundred or so ABA analogues synthesized so far have suggested that any modification would reduce the activity,[18] but it should be highlighted that there are few analogues that were specifically designed to strengthen the activity. Investigation of their biological activity and metabolism should not only provide the means for the practical use of ABA but also give important information about the properties of the ABA receptor and metabolic enzymes.

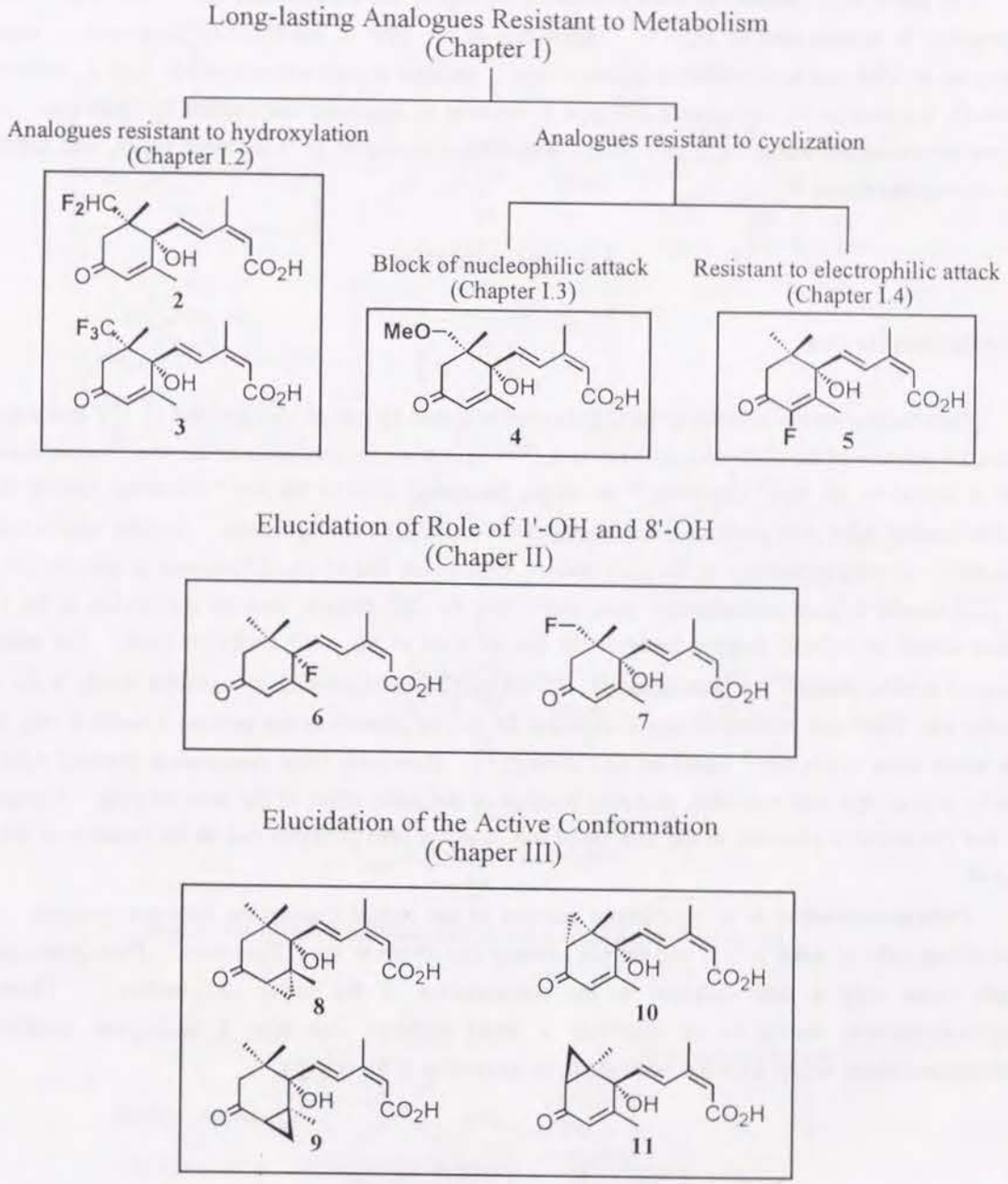


Fig. 2. New ten analogues of ABA synthesized in this study.

Chapter I

Long-Lasting Analogues of Abscisic Acid Resistant to Metabolism

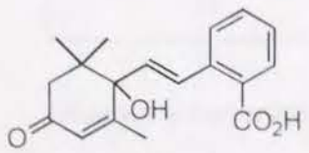
The inactivation pathway of ABA is illustrated in Fig. 3, and the structural part of the ABA molecule responsible is summarized in Fig. 4. According to the type of inactivation, long-lasting, resistant analogues of ABA can be classified as follows: type 1, resistant to photoisomerization; type 2, resistant to metabolic inactivation by conjugation and type 3, resistant to metabolic inactivation by oxidation. This chapter considers the design of highly potent, long-lasting analogues of these three types, and describes four analogues of type 3.

I.1 Introduction

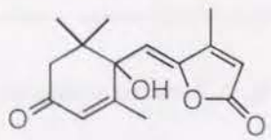
Photoisomerization

Photoisomerization of ABA to the (2*E*)-isomer is caused by natural sunlight and by UV irradiation to give a 1:1 mixture of the (2*Z*)- and (2*E*)-isomers.[19-21] *Cis-trans* isomerization of the C-C double bond by light is caused by the π-π^* transition,[20] so raising the energy level of the π-π^* transition state at the 2-double bond of ABA may protect against isomerization to the (2*E*)-configuration. Another way would be to increase the relative stability of the (2*Z*)-isomer, or decrease that of the (2*E*)-isomer at equilibrium. If the (2*Z*)-isomer is thermodynamically more stable than the (2*E*)-isomer, then its conversion to the (2*E*)-isomer would be reduced independently of the energy level of the π-π^* transition state. No analogue designed in either manner has been reported. Fixing the (2*Z*)-configuration by covalent bonds is the more reliable way to prevent isomerization. Compound **25** and its derivatives that possess a benzene ring in the side chain were synthesized based on this strategy.[22] However, these compounds showed ABA-like activity only at high concentration, probably because of the steric effect of the benzene ring. Compound **26** that possesses a γ-lactone in the side chain was inactive, also probably due to the absence of the free acid.[23]

Photoisomerization is an equilibrium reaction of the mutual conversion between isomers. The equilibrium ratio of ABA is 1:1, and the (2*E*)-isomer can return to the (2*Z*)-isomer. Photoisomerization would cause only a little decrease in the concentration of the active (2*Z*)-isomer. Therefore, photoisomerization seems to be relatively a small problem and type 1 analogues resistant to photoisomerization would have the small effect on increasing in the activity.



25



26

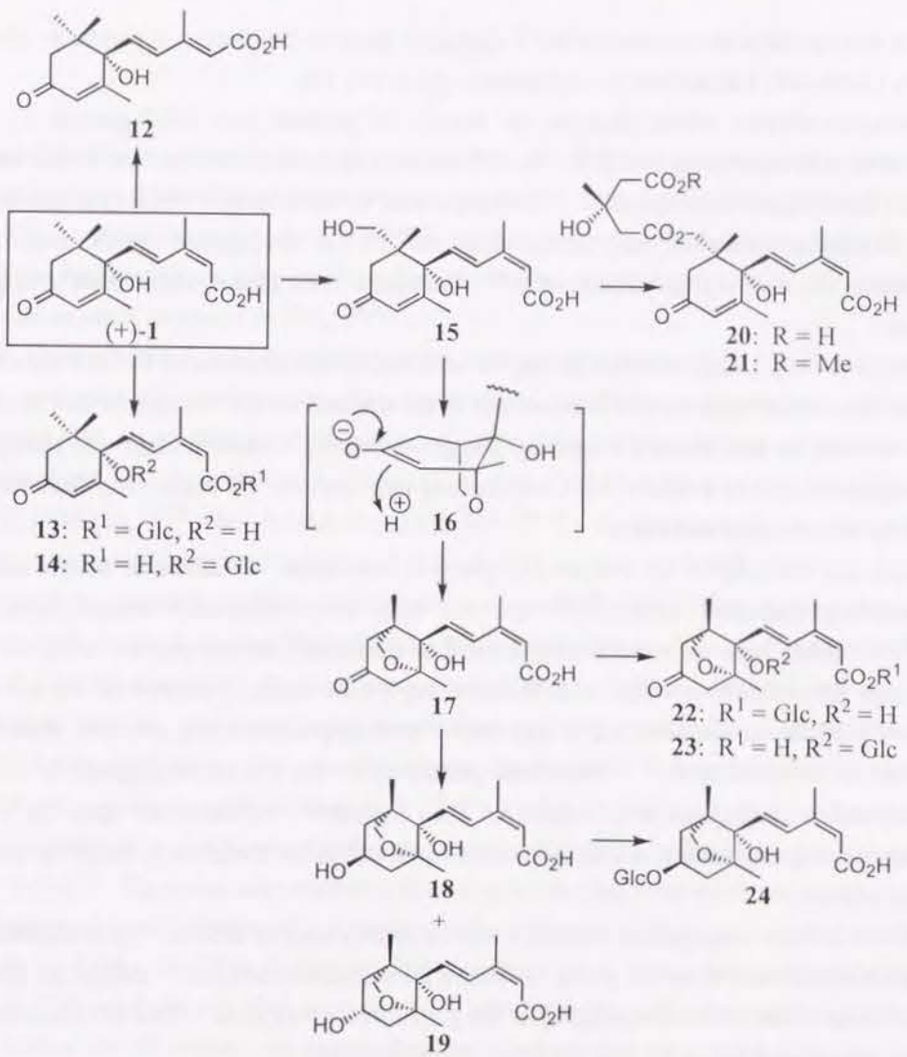


Fig. 3. Inactivation pathway of (+)-ABA (**1**) (Glc: β-D-glucose).

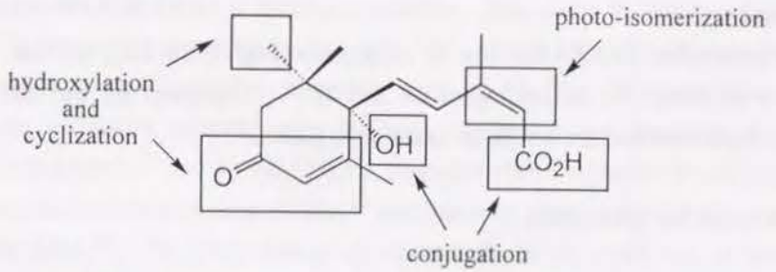


Fig. 4. Structural parts of ABA responsible for the short-life.

Metabolic Inactivation by Conjugation

ABA is conjugated at the 1-carboxyl and 1'-hydroxyl groups with glucose in plants, to give the 1-*O*-glucosyl ester (ABA-GE, **13**) and the 1'-*O*-glucoside (ABA-GS, **14**).

A glucosyltransferase which catalyzes the transfer of glucose from UDP-glucose to ABA with formation of ABA-GE has been purified.[24] The pH optimum of this enzyme was 5.0, so the formation of ABA-GE may be catalyzed in the vacuole. This is supported by the findings of Lehmann and Glund, who located the conjugated metabolites only in the vacuoles.[25] The conjugation would mean irreversible compartmentation into the vacuoles of plant cells.[25] The nature of the glucosyltransferase giving ABA-GS is not known.

Resistance to conjugating to ABA-GE may be achieved by esterification of the C-1 carboxyl group. However, the absence of ABA methyl ester activity in the stomatal assays[26] suggests that the free acid is required for activity, so esterification would not strengthen activity. Methyl, ethyl and phenyl esters of ABA had comparable activity to that of ABA only in long-term assays,[27-29] suggesting that these activities were caused by the release of free ABA.

The chemical reactivity of the 1'-hydroxyl group is very poor; it cannot be acetylated under any reaction conditions attempted to date[30,31] and can only trimethylsilylated using bis(trimethylsilyl) acetamide.[32,33] It has been assumed that the steric[30] or electronic[31] effects prevent reactions of the 1'-hydroxyl group; the steric effect would be attributed to the 1'-side chain, 2'-methyl (C-7') and 6'-methyls (C-8' and C-9'), while the electronic effect may derive from its position being activated electronically by both the enone and dienoic acid.[34] Therefore, protection of the 1'-hydroxyl group is difficult, and analogues resistant to conjugation at C-1' have not been reported. As described later, the 1'-hydroxyl group is a significant group for activity, so its protection, as well as the 1-carboxyl, would be inefficient for an increase in activity.

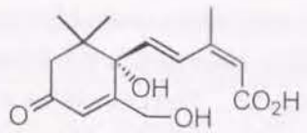
Resistance to these conjugations without a loss of activity may be afforded by modification of sites other than the carboxyl and hydroxyl group, to inhibit the enzymatic catalysis. Design of this analogue requires knowledge of the substrate specificity of the glucosylating enzymes. This is unknown, although the specificity seems to be less strict than that of the 8'-hydroxylase.

A different ratio between the conjugations at C-1 and at C-1' has been found in the different plants and tissues.[35] The conjugation pathway to ABA-GE and ABA-GS seems to be a non-specific removal mechanism for unwanted acids.[36] Therefore, type 2 analogues resistant to the conjugation may not be applied to various plants.

The oxidized metabolites of ABA can also be conjugated (Fig. 3, **20**-**24**), but this is not related to regulation of the activity except for the conjugates **20** and **21**. Conjugates **20** and **21** are metabolites specific to *Robinia pseudacacia* and are not found in the other plants.[37]

Metabolic Inactivation by Oxidation

The metabolic oxidation of ABA in plants is initiated by hydroxylation at C-8' to produce 8'-HOABA (**15**),[2,36] as shown in Fig. 3. Gillard and Walton in 1976, found 8'-hydroxylating activity with high-



27

substrate specificity for (+)-ABA in the particulate portion of a cell-free enzyme system from the liquid endosperm of immature fruits of the Eastern Wild cucumber.[38] This required NADPH and O_2, and was inhibited by CO, so it was considered to be a microsomal cytochrome P-450 monooxygenase.[39] This was supported by the finding that one atom of ^{18}O is incorporated into the 8'-oxygen of PA in leaves and roots of *Xanthium strumarium* incubated in $^{18}O_2$.[40,41]

The 8'-hydroxylase of ABA is believed to be induced by ABA itself,[42] but it has not yet been isolated and its detailed characteristics including the substrate specificity have remained unclear. Some investigations of ABA metabolism in cell free systems, cell cultures and tissues have revealed that (±)-ABA is partly metabolized to 7'-hydroxy-ABA (7'-HOABA, **27**),[43,44] (-)-ABA to (-)-7'-HOABA,[45-47] (+)-ABA to (+)-7'-HOABA,[47,48] and (-)-ABA to (+)-PA.[38,47,49,50] Furthermore, (+)-7'-HOABA has been isolated as an endogenous compound in leaves of *Vicia fava*.[51] When (-)-7'-HOABA was identified as a metabolite of feeding unnatural (-)-ABA to *Xanthium strumarium* in 1986,[46] this finding was rationalized by the fact that ABA is pseudo-symmetrical about the plane that intersects C-1'–C-4' and contains the side chain; C-7' of (-)-ABA can occupy the space occupied by C-8' of (+)-ABA when the ring adopts the less favored half-chair with the side chain being pseudo-equatorial (the conformation of ABA described in Chapter III). It seemed that there is no specific enzyme catalyzing the hydroxylation at C-7' of unnatural (-)-ABA in plants, so one enzyme oxidizes both C-8' of (+)-ABA and C-7' of (-)-ABA. The steric specificity of this enzyme is also involved in the conformational change of ABA to the less favored form in binding to proteins.[52] However, conversions to the minor metabolites were much influenced by the tested tissues and cells, so it is unlikely that only 8'-hydroxylase oxidizes all of C-8' and C-7' in (+)-ABA and C-7' and C-8' in (-)-ABA. In 1994, further detailed investigations in maize suspension-cultured cells by Balsevich *et al.*[53] showed that (+)-ABA is oxidized to (-)-PA inside the cell, whereas (-)-ABA is converted to (-)-7'-HOABA at the cell surface, suggesting that the 7'-hydroxylase of (-)-ABA is not the same as the 8'-hydroxylase of (+)-ABA. These findings suggest that the four hydroxylations are catalyzed by two or more enzymes. The exact specificity of the 8'-hydroxylase must be investigated using purified and isolated enzymes.

The first metabolite 8'-HOABA is extremely unstable. Ever since Milborrow identified 8'-HOABA as "Metabolite C" in tomato plants supplied with (±)-[2-^{14}C]-ABA in 1968,[54] it has not been isolated from plant extracts because it spontaneously cyclizes *in vitro* to PA.[36] The existence of this unstable intermediate in the metabolic pathway was indirectly confirmed by detection of acetylated[32] and trimethylsilylated compounds,[55] and by isolating the conjugate with β-hydroxy-β-methylglutaric acid (**20**, **21**) from immature seeds of *Robinia pseudacacia*,[37] and that with glucose from sunflower leaves fed with the methyl ester of ABA.[52] In 1995, Zou *et al.* reported that 8'-HOABA can be isolated as a borate complex by heating PA and boric acid in glacial acetic acid, and suggested that 8'-HOABA is active in lipid and oleosin biosynthesis.[56]

The cyclization of 8'-HOABA to PA is an intramolecular Michael addition which is initiated with the nucleophilic addition of the 8'-oxygen to the electron-deficient 2'-carbon and completed by subsequent protonation at C-3' in the intermediate enolate **16**. This protonation *in vivo* occurs stereospecifically from the α-face of the cyclohexanone ring (the *si*-face of C-3' in **16**), so the cyclization to PA is believed to proceed enzymatically *in vivo*.[57] PA is inactive in many bioassay systems.

PA *in vivo* is reduced at the 4'-carbonyl group to give further inactive dihydrophaseic acid (DPA, **18**) and *epi*-DPA (**19**).[2,36] PA-reducing activity has been found in the soluble portion of a cell-free enzyme system.[38] The inactivation of ABA to PA and DPA is inhibited by plant growth retardants of the tetcyclacis and triazole type which are inhibitors of cytochrome P-450.[58,59] The further metabolism of ABA is unknown, although the side chain seems to be degraded.

This oxidation pathway causes the intrinsic inactivation of ABA and it is common among higher plants.[2-4,6,36] Type 3 analogues resistant to this metabolism would be more effective in various plants than the types 1 and 2. Thus, the author selected type 3 as the target for long-lasting analogues of ABA. Since 8'-HOABA probably remains active, the analogues should not be metabolized to PA. Therefore, the type 3 analogues were designed using two strategies based on the above mechanism of oxidative inactivation. One was the blockage of 8'-hydroxylation and the other was the cyclization of 8'-HOABA to PA. There are two ways to design the latter type: one is by lowering the nucleophilicity of the 8'-oxygen and the other is by lowering the electrophilicity of the 2'-carbon.

I.2 8',8'-Difluoro- and 8',8',8'-Trifluoroabscisic Acids as the 8'-Hydroxylation-Resistant Analogues

Design Concept

Hydroxylation at C-8' by cytochrome P-450 would include hydrogen abstraction by the activated oxygen and recombination of the resulting carbon and hydroxyl radicals.[60] The most effective chemical modification that can confer resistance to this radical oxidation is the introduction of fluorine atoms at C-8'.

The physical properties of fluorine and fluorocarbons are shown along with those of hydrogen and oxygen in Table 1.[61] The strength of the C-F bond is higher than that of the C-H bond, meaning that the C-F bond is more stable to radical cleavage than the C-H bond. The resulting C-F radical tends to act as an electrophilic radical because of the strong electronegativity of fluorine, so it has low reactivity with the hydroxy radical, which is electrophilic. Additionally, its compactness is another reason for using fluorine. The van der Waals radius of fluorine is the shortest next to that of hydrogen (1.13-fold of that of hydrogen), and except for monofluorocarbons, the length of the C-F bond is about 1.2-fold that of the C-H bond. This means that introducing fluorines induces resistance to metabolic oxidation without much influence on the steric size of the molecule; that is, it retains affinity for the receptor.

The introduction of fluorines into many bioactive molecules[62-68] and various methods of fluorination[69-71] have been reported. Rose *et al.*,[72] have synthesized 7',7'-difluoro-ABA (**28**), but it did not strengthen the activity of parent molecule because as described above, C-7' is the site of oxidation of

Table 1. Physicochemical Properties of H, F and O

a) Electronegativity and Van der Waals Radius of H, F and O

	H	F	O
Electronegativity	2.1	4.0	3.5
van der Waals radius (Å)	1.2	1.35	1.4

b) Bond Strength (kcal mol^{-1}) of R-H, R-F and R-O

R	H	F	O
CH_3	105	108	92
CH_2F	101	-	-
CHF_2	103	-	-
CF_3	107	131	-

c) Bond Length (Å) of R-H, R-F and R-O

R	H	F	O
CH_3	1.09	1.83	1.43
CH_2F	-	1.33	-
CF_3	1.10	-	-
CH_2=CH	1.07	1.32	-

unnatural (1'*R*)-(-)-ABA and is the minor site for that of natural (1'*S*)-(+)-ABA.[43-53] Replacing hydrogens with fluorines at C-8' which is the major site of oxidation, can block hydroxylation at C-8' without reducing affinity for the active site on the uptake carrier and receptor. This renders lasting activity. Therefore, 8',8'-difluoro-ABA (**2**) and 8',8',8'-trifluoro-ABA (**3**) were designed as analogues that can resist 8'-hydroxylation.

Results and Discussion

Synthesis and identification

Difluoro-ABAs were synthesized by a modification of the method reported for the synthesis of (±)-methyl phaseate[73] (Fig. 5). Hydroxymethylation of 2,6-dimethyl-1-cyclohexanone (**29**) by the method of Grieco and Hiroi[74] gave hydroxymethyl ketone **30**. Oxidation of **30** gave the formyl ketone **31**. Compound **31** was fluorinated using diethylaminosulphur trifluoride (DAST) to give the difluoromethyl ketone **32** in a 45% yield. The reaction of **32** with alkynyl lithium gave the tetrahydropyranyl (THP) ether **33**. Deprotection of **33** gave the acetylenic diol **34**, which was then acetylated to afford the acetylenic acetate **35**. Dehydration of **35** gave the enyne acetate **36**. Reduction of **36** gave the dienol **37**, which was then oxidized yielding the dienone **38**. A Wittig reaction with **38** gave the methyl ester **39** as a mixture of (2*Z*)- and (2*E*)-isomers. Bromination of **39** and then dehydrobromination formed the didehydro compound **40**, which on photosensitized oxygenation and subsequent treatment with basic alumina gave the methyl esters of difluoro-ABAs: four stereoisomers resulting from the 2*Z*/2*E* isomerism and from the *cis* or *trans* relationship of the 6'-difluoromethyl group to the 1'-hydroxyl group. Hydrolysis of these methyl esters gave an isomeric mixture of (±)-8',8'-difluoro-ABA (**2**) and its (±)-(2*E*)-isomer (**41**), and of (±)-9',9'-difluoro-ABA (**42**) and its (±)-(2*E*)-isomer (**43**) (*ca* 1:2:4:8, as determined by HPLC). This mixture was separated into its components by HPLC with an ODS column.

Trifluoro-ABAs were synthesized from compound **44** prepared by the method reported for the synthesis of 16,16,16-trifluororetinal[75] (Fig. 6). In the same manner as for **39**, compound **44** gave an

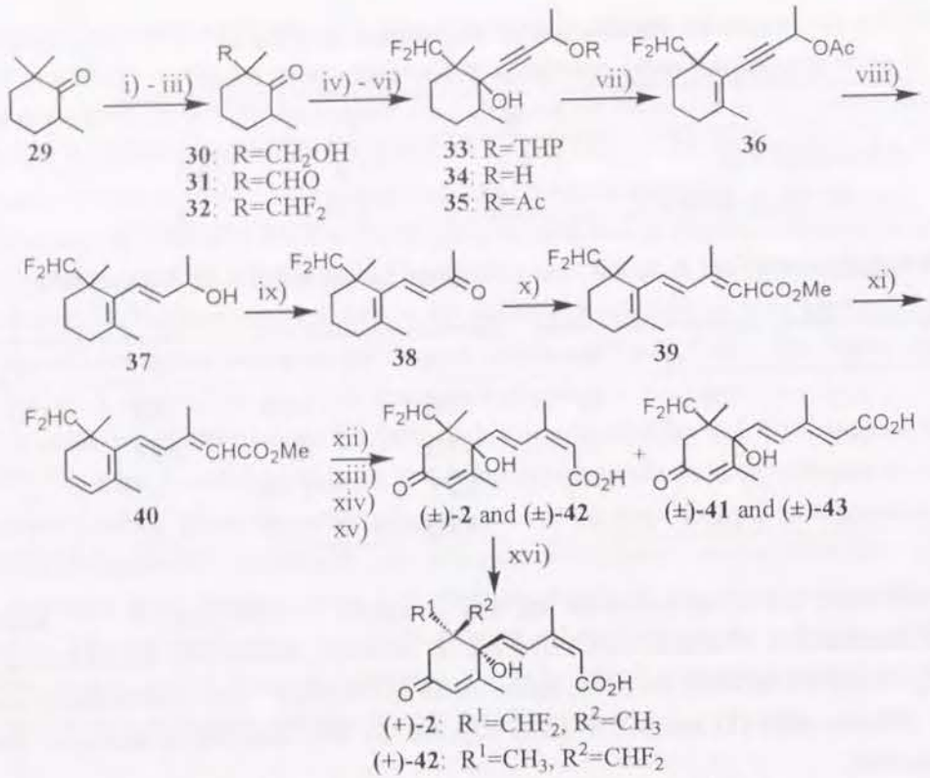


Fig. 5. Synthesis and optical resolution of **2** and **42**.

i) LDA, HCHO ii) PCC iii) DAST iv) Li≡OTHP v) pyridinium *p*-TsOH vi) Ac_2O, pyridine vii) $POCl_3$, pyridine viii) Red-Al ix) MnO_2 x) $Ph_3P{=}CHCO_2Me$ xi) a) NBS, BPO b) quinoline xii) rose bengal, O_2, hv xiii) basic alumina xiv) NaOH xv) ODS HPLC xvi) HPLC with Chiralcel OD.

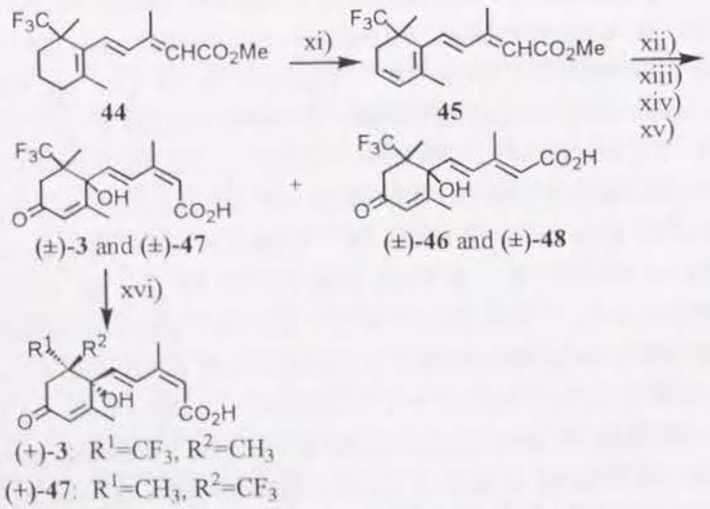


Fig. 6. Synthesis and optical resolution of **3** and **47**.
For xi) - xvi), see legends of Fig. 5.

isomeric mixture of (±)-8',8',8'-trifluoro-ABA (**3**) and its (±)-(2*E*)-isomer (**46**), and of (±)-9',9',9'-trifluoro-ABA (**47**) and its (±)-(2*E*)-isomer (**48**) (*ca* 1:2:4:8, as determined by HPLC). This mixture was separated into its components by HPLC with an ODS column.

The ratio of the C-8' fluorinated analogue to C-9' fluorinated analogue in difluoro- and trifluoro-ABAs was 1:4, which was brought about during the photosensitized oxygenation of the didehydro compounds (**40** and **45**). This ratio of 1:4 is believed to result from the stereoselective addition of a singlet oxygen from the sterically or electrically less-hindered site, that is, the opposite side of the di- and trifluoromethyl groups.

Identification of difluoro- and trifluoro-ABAs was accomplished by analysis of ^{1}H NMR and ^{13}C NMR spectral data. In the ^{1}H NMR spectra, the 6'-methyl groups of (±)-**2** and -**3** (δ 1.10 and 1.26) appeared in a higher field than those of (±)-**42** and -**47** (δ 1.18 and 1.33), respectively. The 9'-protons of ABA (δ 1.01) appeared at higher field than the 8'-protons (δ 1.11),[76] so the spectra of (±)-**2** and -**3** both lacked a methyl singlet corresponding to the 8'-protons of ABA, and those of (±)-**42** and -**47** lacked a methyl singlet corresponding to the 9'-protons. Analogues (±)-**2** and -**42** both showed a triplet signal of one proton at δ 6.03 and 5.92, respectively, which were assigned to the proton at C-8' and at C-9', respectively, bonded to two fluorine atoms. These findings showed that (±)-**2** was (±)-8',8'-difluoro-ABA and (±)-**3** was (±)-8',8',8'-trifluoro-ABA, and that (±)-**42** was (±)-9',9'-difluoro-ABA and (±)-**47** was (±)-9',9',9'-trifluoro-ABA. The ^{13}C NMR spectra confirmed the above identification. The signals of C-8' of (±)-**2** and C-9' of (±)-**42** appeared as triplets at δ 119.9 and 119.2, respectively, by coupling with two fluorine atoms, and the ^{13}C signals of C-8' of (±)-**3** and C-9' of (±)-**47** appeared as quartets at δ 130.3 and 129.1, respectively, by coupling with three fluorine atoms.

(±)-Difluoro- and (±)-trifluoro-ABAs were optically resolved by HPLC with a Chiralcel OD column to afford the (+)- and (-)-enantiomers with an optical purity of more than 99%. The CD spectra of the (+)-enantiomers showed the same positive first and negative second Cotton effects, i.e. the positive exciton chirality, as those of (*S*)-(+)-ABA.[77] Therefore, the absolute configuration at C-1' of (+)-difluoro- and (+)-trifluoro-ABAs is *R*, while that at C-6'is *S* for (+)-**2** and -**3**, and *R* for (+)-**42** and -**47**.

Biological activity

The optically active analogues were compared with the (+)- and (-)-ABAs and (-)-PA for their inhibitory activity in four bioassays: lettuce seed germination[78]; elongation of the second leaf sheath of rice seedlings[79]; α-amylase induction by gibberellin A_3 in barley half-seeds without embryos[80]; and stomatal opening of the epidermal strips of spiderwort.[81] Values for the concentration giving half-maximal inhibition (IC_{50}) from the assays are summarized in Table 2. (2*E*)-isomers of racemic **2**, **3**, **42** and **47** were inactive in the assays (data not shown).

In the rice elongation assay, the C-8' trifluorinated analogue (+)-**3** was extremely powerful; its IC_{50} value was only 0.082 μM, while that for (+)-ABA was 2.6 μM, meaning (+)-**3** was more than 30 times as effective as (+)-ABA. In the lettuce germination assay, (+)-**3** caused 50% inhibition of germination at a concentration of 1.9 μM, while (+)-ABA caused the same degree of inhibition at 5 μM; i.e. the activity of (+)-**3** was 2.6 times that of (+)-ABA. The C-8' difluorinated analogue (+)-**2** also showed strong activity in the elongation and germination assays; *ca* six and two times those of ABA, respectively. Investigation

Table 2. The IC_{50} Values for Optically Active ABA, **2**, **3**, **42** and **47**

Compound	IC_{50} in assay			
	Rice seedling elongation (μM)	Lettuce seed germination (μM)	Barley α-amylase induction (μM)	Spiderwort stomatal opening (nM)
(+)-ABA	2.6	5.0	2.9	5.0
(+)-**2**	0.45	2.3	3.3	5.8
(+)-**3**	0.082	1.9	2.0	4.6
(+)-**42**	1.5	5.7	4.0	4.3
(+)-**47**	2.4	7.9	6.4	4.2
(-)-ABA	3.5	9.7	7.3	67
(-)-**2**	4.0	10	7.5	68
(-)-**3**	2.8	11	24	330
(-)-**42**	12	12	26	62
(-)-**47**	27	7.9	20	60
(-)-PA	>300	>300	9.2	>1000

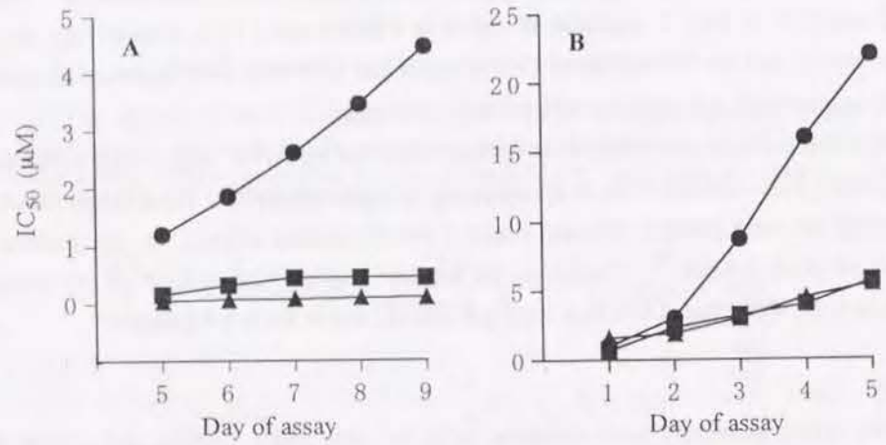


Fig. 7. Changes with time in the inhibitory activity of (+)-**1**, -**2**, and -**3** in the rice seedlings elongation (A) and the lettuce seed germination (B).
●, (+)-**1**; ■, (+)-**2**; ▲, (+)-**3**.

of the changes with time in the effects of (+)-**2** and -**3** in these two assays showed that (+)-**2** and -**3** were superior in stability to (+)-ABA (Fig. 7); as time passed, the ratio of the activity of (+)-**2** and -**3** to that of (+)-ABA increased, becoming 6/1 on day 5 and 10/1 on day 9 for (+)-**2**, and 25/1 on day 5 and 40/1 on day 9 for (+)-**3** in the elongation assays, and 2/1 on day 2 and 4/1 on day 5 for both analogues in the germination assay. These results suggest that the high activity of (+)-**2** and -**3** observed in these two assays resulted from a delayed inactivation, as expected. The result that the long-lasting effect by the 8'-fluorination was more remarkable in the rice assay than in the lettuce assay may be explained by the

demonstration of Orlandini *et al.* that the major metabolic pathway in lettuce seeds is the conjugation to ABA-GE, not the oxidation to PA.[82]

In the α-amylase and stomata assays, the activities of (+)-**2** and -**3** were as effective as those of (+)-ABA. In the α-amylase assay which takes place over a rather long period (2 days), (-)-PA showed activity *ca* 1/3 that of (+)-ABA. This result agrees with those reported by Lin and Ho.[83] This finding implies that blockage of conversion to (-)-PA via 8'-HOABA does not contribute to the enhancement of the activity, thus explaining the lower activity of (+)-**2** and -**3** in the α-amylase assay than in the elongation assays. In the stomata assay which is a short-term assay (3 hr), the activity of a test compound will be unaffected by the speed of its metabolism.[84] In this assay, therefore, blockage of metabolic inactivation will have little or no influence on the activity. Consequently, the results obtained in these four assays suggests that (+)-**2** and -**3** act as longer-lasting analogues, as designed, although metabolism of (+)-**2** and -**3** was not examined in this study. The difluoromethyl group can act as a functional group that inactivates the cytochrome P-450 enzyme,[85] so the difluorinated analogue (+)-**2** can act as a suicide inhibitor of the 8'-hydroxylase. However, the outward appearance of tested plants suggested that (+)-**2** as well as (+)-**3** and (+)-ABA was non-toxic.

The activities of the C-9' difluorinated analogue (+)-**42** and the C-9' trifluorinated analogue (+)-**47** were almost equal to that of (+)-ABA in all the bioassays. This result suggests that di- and trifluorination of C-9' of (+)-ABA did not influence the affinity for the active site on the receptor and had no effect on inhibiting the approach or action of the 8'-hydroxylation enzyme.

The effect of (-)-ABA varied according to the assays tested. In the rice elongation assay, (-)-ABA was effective almost equally with (+)-ABA. There has been no report about the effects of the isolated (-)-ABA on the rice elongation assay so far, and (-)-ABA was believed to be inactive based on that (+)-ABA was twice as effective as (±)-ABA.[86] This study found firstly that (-)-ABA is potent in the rice assay. In the lettuce and α-amylase assays, (-)-ABA showed about 1/2 activity of (+)-ABA, while the effect of (-)-ABA on the stomatal assay was 1/10 that of (+)-ABA. This indicates that (-)-ABA is more potent in the assays affected more largely by metabolic stability of a tested compound. (-)-ABA may have a smaller affinity for the active site on the receptor than (+)-ABA does, but delay of metabolic inactivation[87-90] seems to contribute to the high activity in the long-term assays, as (+)-**2** and -**3**. The (-)-enantiomers of the di- and trifluoro analogues showed activity which was the same as or less than that of (-)-ABA; compound (-)-**2** was equivalent to (-)-ABA in all the assays, (-)-**3** was less active in the α-amylase and stomata assays, and (-)-**42** and -**47** were less effective in the elongation and α-amylase assays. The cyclohexenone ring of ABA is pseudo-symmetrical, so (-)-ABA is considered to bind to the same site that (+)-ABA binds.[91] In this case, C-8' or C-9' of (-)-ABA occupies the site normally filled by C-7' of (+)-ABA[91] which is essential for activity.[92,93] The van der Waals radius of fluorine is a little larger than that of hydrogen (Table 1). The slightly bulkier C-8' or C-9' of the (-)-enantiomers of the long-lasting analogues would cause a weakening of the affinity for the active site and hence reduced activity as observed. This implies that the steric requirement around C-2' is much stricter than that around C-6'. The differences in the effects of the individual (-)-enantiomers among the four assays suggests that steric tolerance of the binding site for the equatorial direction and for the axial direction at C-2' on the β-face of the ring differs with species or tissues.

Experimental

General procedures

^{1}H NMR and ^{13}C NMR spectra were recorded with TMS as the internal standard using a Jeol GX400 (400 MHz), Jeol GSX270J (270 MHz) and JNM GSX-500 (500 MHz). For clarity, the conventional ABA numbering system is used in the assignment of peaks in the ^{1}H NMR and ^{13}C NMR spectra. Mass spectra were obtained with a Jeol JMS-DX300/DA5000 mass spectrometer. GC-MS was conducted with a 1% OV-17 column (1 m x 2.6 mm) in the EI mode. CD spectra and optical rotation were recorded with Jasco J-600 spectropolarimeter and DIP-4 or DIP-370 digital polarimeter, respectively.

(±)-*2-Hydroxymethyl-2,6-dimethyl-1-cyclohexanone* (**30**)[73]

To a solution of diisopropylamine (35.4 g) in THF (100 ml) at -75°C was added *n*-butyl lithium (*n*-BuLi) (a 1.6 M solution in hexane, 218 ml). After 20 min, 2,6-dimethyl-1-cyclohexanone (**29**, 40 g) was added dropwise. The reaction mixture was warmed to -25°C, and gaseous formaldehyde, which was generated from paraformaldehyde (60 g) by heating the mixture at 170-190°C, was passed into the reaction mixture in a stream of nitrogen. The reaction mixture was stirred for 60 min at -25 to -15°C and then warmed to room temperature. After being quenched with saturated NH_4Cl, the mixture was extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (9:1-7:3) to afford 31.5 g (64% yield) of **30** as a mixture of two diastereomers (59:41, determined by GC analysis). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (3H, d, J = 6.4 Hz, Me-6), 1.03 (3H, d, J = 6.4 Hz, Me-6), 1.09 (3H, s, Me-2), 1.20 (3H, s, Me-2), 1.28-2.10 (12H, m, H-3, H-4, H-5), 2.63 (1H, m, H-6), 2.65 (1H, m, H-6), 3.44 (1H, d, J = 11.6 Hz, OCH_2), 3.51 (1H, d, J = 11.6 Hz, OCH_2) 3.58 (1H, d, J = 10.7 Hz, OCH_2), 3.92 (1H, d, J = 10.7 Hz, OCH_2); GC-MS *m/z* (rel. int.): 156 $[M]^+$ (3), 138 (23), 126 (18), 111 (14), 95 (22), 83 (100), 69 (46).

(±)-2-*Formyl*-2,6-*dimethyl*-1-*cyclohexanone* (**31**)

A mixture of **30** (66 g) and pyridinium chlorochromate (110 g) in CH_2Cl_2 (200 ml) was stirred at room temperature for 2 hr. The suspension was filtered, and the filtrate was then concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (9:1) to give **31** (33 g, 51% yield) as a mixture of two diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (3/2H, d, J = 6.7 Hz, Me-6), 1.06 (3/2H, d, J = 6.4 Hz, Me-6), 1.21 (3/2H, s, Me-2), 1.35 (3/2H, s, Me-2), 1.35-2.46 (6H, m, H-3, H-4, and H-5), 2.52 (1/2H, m, H-6), 2.65 (1/2H, m, H-6), 9.45 (1/2H, d, J = 0.6 Hz, CHO), 9.72 (1/2H, s, CHO); GC-MS *m/z* (rel. int.): 154 $[M]^+$ (4), 139 (4), 126 (57), 111 (35), 97 (34), 84 (32), 71 (100).

(±)-2-*Difluoromethyl*-2,6-*dimethyl*-1-*cyclohexanone* (**32**)

Compound **31** (33 g) was added dropwise to a solution of DAST (57.4 g) in CH_2Cl_2 (300 ml) cooled to -78°C under N_2. The mixture was then warmed to room temperature and stirred for 4 hr. After being quenched with saturated $NaHCO_3$ and H_2O, the mixture was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (97:3) to give **32** (17.1 g, 45% yield) as a mixture of two diastereomers.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01 (3/2H, d, J = 6.4 Hz, Me-6), 1.07 (3/2H, d, J = 6.4 Hz, Me-6), 1.13 (3/2H, t, $^4J_{HF}$ =1.2 Hz, Me-2), 1.29 (3/2H, t, $^4J_{HF}$ =1.2 Hz, Me-2), 1.34-2.27 (6H, m, H-3, H-4, and H-5), 2.58 (1/2H, m, H-6), 2.61 (1/2H, m, H-6), 5.98 (1/2H, t, $^2J_{HF}$ = 56.2 Hz, CHF_2), 6.03 (1/2H, t, $^2J_{HF}$ = 55.5 Hz, CHF_2); GC-MS *m/z* (rel. int.): 176 $[M]^+$ (30), 128 (11), 113 (8), 109 (8), 98 (37), 86 (25), 81 (11), 73 (17), 69 (100).

(±)-4-(1'-*Hydroxy*-2'-*difluoromethyl*-2',6'-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*-*THP ether* (**33**)

A 1.6 M solution of *n*-BuLi in hexane (100 ml) was added dropwise to a stirred solution of 1-methyl-2-propynyl THP ether (24 g) in THF (100 ml) over 30 min at -78°C under N_2. After being stirred for 1 hr, the reaction mixture was warmed to -25°, and **32** (17.1 g) in THF (50 ml) was added dropwise to the stirred mixture. The mixture was stirred for 2 hr at -25 to -10°C and then warmed to room temperature. After being quenched with 0.1 M NH_4Cl (250 ml), the mixture was extracted with Et_2O, and the organic layer was successively washed with 0.1 M NH_4Cl and H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (5:1) to give **33** (24.6 g, 77% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) of the major diastereomer: δ 1.06 (3H, d, J = 6.4 Hz, Me-6'), 1.18 (3H, s, Me-2'), 1.48 (3H, d, J = 6.7 Hz, H-1), 1.51-2.11 (12H, m, H-3', H-4', H-5', H-2", H-3", and H-4"), 4.62 (1H, q, J = 6.7 Hz, H-2), 4.94 (1H, dd, J =4.3 and 2.7 Hz, H-1"), 6.06 (1H, t, $^2J_{HF}$ = 56.2 Hz, CHF_2); EIMS *m/z* (rel. int.): 330 $[M]^+$ (3), 246 (21), 228 (30), 210 (31), 177 (15), 159 (48), 139 (23), 121 (66), 109 (41), 91 (43), 84 (100).

(±)-4-(1'-*Hydroxy*-2'-*difluoromethyl*-2',6'-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*. (**34**)

To a stirred solution of **33** (24.6 g) in EtOH (400 ml) was added pyridinium *p*-toluenesulfonate (2 g), and the mixture was stirred at 55°C for 5 hr. The solution was concentrated and the residue was diluted with Et_2O (1 litre), successively washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. Chromatography of the residual oil on silica gel with hexane-EtOAc (5:1) gave **34** (17.8 g, 97% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) of the major diastereomer: δ 1.05 (3H, d, J = 6.4 Hz, Me-6'), 1.13 (3H, s, Me-2'), 1.22-2.10 (6H, m, H-3', H-4', and H-5'), 1.49 (3H, d, J = 6.7 Hz, H-1), 4.61 (1H, q, J = 6.7 Hz, H-2), 6.07 (1H, t, $^2J_{HF}$ = 56.2 Hz, CHF_2); EIMS *m/z* (rel. int.): 246 $[M]^+$ (60), 228 (4), 213 (7), 193 (8), 177 (6), 166 (28), 149 (15), 139 (81), 121 (100), 109 (80).

(±)-3-(1'-*Hydroxy*-2'-*difluoromethyl*-2',6'-*dimethylcyclohexyl*)-1-*methyl*-2-*propynyl acetate* (**35**)

A solution of **34** (17.8 g) and Ac_2O (40 ml) in pyridine (100 ml) was stirred at room temperature for 13 hr. The solution was poured into ice-cooled H_2O and extracted with Et_2O. The organic layer was successively washed with 0.1 N HCl, saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (9:1) to give **35** (17.8 g, 85% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) of the major diastereomer: δ 1.04 (3H, d, J = 6.4 Hz, Me-6'), 1.09-1.95 (6H, m, H-3', H-4', and H-5'), 1.12 (3H, s, Me-2'), 1.52 (3H, d, J = 6.7 Hz, Me-1), 2.07 (3H, s, OAc), 5.45 (1H, q, J = 6.7 Hz, H-1), 6.06 (1H, t, $^2J_{HF}$ = 57.2 Hz,

CHF_2); EIMS *m/z* (rel. int.): 288 $[M]^+$ (13), 270 (3), 246 (11), 228 (63), 213 (13), 193 (11), 177 (16), 166 (21), 135 (35), 121 (29), 109 (37), 93 (18), 80 (100).

(±)-3-(2'-*Difluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-1-*methyl*-2-*propynyl acetate* (**36**)

To a stirred solution of **35** (17.3 g) in pyridine (150 ml), a mixture of $POCl_3$ (37.3 ml) and pyridine (40 ml) was added dropwise at 0°C, and the solution was then heated at 100°C for 23 hr. The solution was poured into ice-cooled H_2O, and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (19:1) to give **36** (5.8 g, 30% yield) as a mixture of two diastereomers. ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (3/2H, t, $^4J_{HF}$ = 1.2 Hz, Me-2'), 1.17 (3/2H, t, $^4J_{HF}$ = 1.2 Hz, Me-2'), 1.37-2.14 (6H, m, H-3', H-4', and H-5'), 1.52 (3H, d, *J* = 6.7 Hz, Me-1), 1.90 (3H, s, Me-6'), 2.07 (3H, s, OAc), 5.57 (1H, q, *J* = 6.7 Hz, H-1), 5.84 (1/2H, t, $^2J_{HF}$ = 57.1 Hz, CHF_2), 5.87 (1/2H, t, $^2J_{HF}$ = 57.1 Hz, CHF_2); EIMS *m/z* (rel. int.): 270 $[M]^+$ (10), 242 (19), 226 (21), 213 (85), 203 (66), 185 (33), 175 (54), 159 (78), 149 (40), 142 (46), 129 (55), 115 (75), 105 (61), 91 (100).

(±)-(E)-4-(2'-*Difluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*ol* (**37**)

To a stirred solution of **36** (4.9 g) in THF (50 ml), a mixture of Red-Al (3.4 M in toluene, 60 ml) and THF (40 ml) was added dropwise at 0°C over 40 min under N_2. The solution was refluxed for 3 hr. A saturated NH_4Cl solution was added to quench the reaction, and the mixture was filtered and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (19:1-9:1) to give **37** (3.4 g, 81% yield) as a mixture of two diastereomers. ^1H NMR (400 MHz, $CDCl_3$): δ 1.09 (3/2H, s, Me-2'), 1.09 (3/2H, s, Me-2'), 1.31 (3/2H, d, $^4J_{HF}$= 6.4 Hz, H-1), 1.31 (3/2H, d, $^4J_{HF}$= 6.4 Hz, H-1), 1.40-2.12 (6H, m, H-3', H-4', and H-5'), 1.71 (3H, s, Me-6'), 4.37 (1H, dq, *J* = 6.4 and 6.4 Hz, H-2), 5.52 (1H, dd, *J* = 16.2 and 6.4 Hz, H-3), 5.66 (1/2H, t, $^2J_{HF}$ = 56.8 Hz, CHF_2), 5.66 (1/2H, t, $^2J_{HF}$ = 56.8 Hz, CHF_2), 5.99 (1H, d, *J* = 16.2 Hz, H-4); EIMS *m/z* (rel. int.): 230 $[M]^+$ (3), 212 (43), 197 (12), 172 (34), 161 (81), 145 (10), 133 (15), 121 (100), 105 (46).

(±)-(E)-4-(6'-*Difluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*one* (**38**)

A mixture of active MnO_2 (27 g) and **37** (3.3 g) was stirred in CH_2Cl_2 (130 ml) at room temperature for 4 hr. The suspension was filtered, and the resulting cake of MnO_2 was washed with CH_2Cl_2. After being concentrated, the residual oil was chromatographed on silica gel with hexane-EtOAc (19:1) to give **38** (2.9 g, 88% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (3H, s, Me-6'), 1.45-2.16 (6H, m, H-3', H-4', and H-5'), 1.80 (3H, s, Me-2'), 2.30 (3H, s, H-1), 5.68 (1H, t, $^2J_{HF}$ = 56.8 Hz, CHF_2), 6.07 (1H, d, *J* = 16.2 Hz, H-3), 7.17 (1H, d, *J* = 16.2 Hz, H-4); EIMS *m/z* (rel. int.): 228 $[M]^+$ (4), 213 (100), 199 (6), 185 (5), 177 (5), 162 (4), 159 (5), 135 (2).

(±)-(2Z,4E *and* 2E,4E)-*Methyl* 5-(6'-*difluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*methyl*-2,4-*pentadienoate* (**39**)

A mixture of **38** (2.9 g) and methyl (triphenylphosphoranylidene)acetate (10 g) was stirred at 175°C for 2 hr, and then dissolved in EtOAc (50 ml). The solution was chromatographed on silica gel with hexane-EtOAc (49:1) to give **39** (3.1 g, 87% yield) as a mixture of two geometrical isomers (2*Z*:2*E* = 45:55, determined by integrating the 6'-methyl singlets in the ^1H NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$): δ 1.12 (3H, s, Me-6'-*E*), 1.16 (3H, s, Me-6'-*Z*), 1.44-2.28 (12H, m, H-3', H-4', and H-5'), 1.73 (3H, d, *J* = 0.6 Hz, Me-2'-*E*), 1.81 (3H, s, Me-2'-*Z*), 2.04 (3H, d, *J* = 1.2 Hz, H-6-*Z*), 2.32 (3H, d, *J* = 1.2 Hz, H-6-*E*), 3.69 (3H, s, CO_2Me-*Z*), 3.72 (3H, s, CO_2Me-*E*), 5.64 (1H, t, $^2J_{HF}$ = 56.8 Hz, CHF_2-*E*), 5.68 (1H, s, H-2-*Z*), 5.73 (1H, t, $^2J_{HF}$ = 56.8 Hz, CHF_2-*Z*), 5.76 (1H, s, H-2-*E*), 6.08 (1H, d, *J* = 15.9 Hz, H-4-*E*), 6.46 (1H, d, *J* = 15.9 Hz, H-5-*E*), 6.50 (1H, d, *J* = 16.5 Hz, H-5-*Z*), 7.61 (1H, d, *J* = 16.5 Hz, H-4-*Z*); EIMS *m/z* (rel. int.): 284 $[M]^+$ (100), 269 (4), 225 (56), 209 (52), 183 (12), 173 (21), 159 (81), 145 (16), 131 (20), 119 (58), 105 (33).

(±)-8',8'-*Difluoro-ABA* (**2**), *its* (2E)-*isomer* (**41**), 9',9'-*difluoro-ABA* (**42**), *and its* (2E)-*isomer* (**43**)

N-bromosuccinimide (NBS) (2.5 g) and benzoyl peroxide (BPO) (26 mg) were added to a solution of **39** (3.1 g) in CCl_4 (20 ml), and the mixture was then refluxed for 2.5 hr under N_2. After cooling the mixture to room temperature, it was filtered, and quinoline (8 ml) was added to the filtrate. The mixture was concentrated, and the residue was heated at 100°C for 1 hr under N_2. After being cooled to room temperature, the reaction mixture was poured into 1% H_2SO_4 (400 ml) and extracted with Et_2O. The organic layer was successively washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel with hexane-EtOAc (99:1-49:1) to give the didehydro compound (**40**, 1.31 g) as a crude oil. A solution of **40** (1.3 g) and rose bengal (0.2 g) in MeOH (200 ml) was stirred under O_2 while being irradiated with a fluorescent lamp at 25°C for 15 hr. After being concentrated, the residue was dissolved in MeOH (20 ml), and alumina (active basic, 15 g) was added to the solution. After evaporating the MeOH, hexane (15 ml) was added to the mixture, and the suspension was stirred at room temperature for 2.5 hr before being chromatographed on alumina. Elution with 40-100% EtOAc in hexane afforded a crude ester as an oil. The crude ester was purified by chromatography on silica gel with hexane-EtOAc (17:3) to give 600 mg (42% yield) of a mixture of four isomers. To a solution of this mixture (600 mg) in MeOH was added 1 N NaOH (10 ml). The mixture was stirred at room temperature for 6 hr, then diluted with H_2O (150 ml) and washed with hexane. The organic layer was discarded, and the saturated layer was acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was separated by HPLC on μBondasphere 5 μ C18-100Å (150 x 19 mm, Waters; solvent, 52% MeOH in 1% AcOH; flow rate, 4.8 ml min^{-1}; detection, 254 nm) to give as amorphous powders 39 mg of (±)-**2**, 57 mg of (±)-**41**, 156 mg of (±)-**42**, and 243 mg of (±)-**43**. (±)-**2**. ^1H NMR (400 MHz, CD_3OD): δ 1.10 (3H, s, H-9'), 1.93 (3H, d, *J* = 1.5 Hz, H-7'), 2.04 (3H, d, *J* = 1.2 Hz, H-6), 2.46 (1H, d, *J* = 17.7 Hz, H-5'-*pro-R*), 2.64 (1H, d, *J* = 17.7 Hz, H-5'-*pro-S*), 5.78 (1H, s, H-2), 5.94 (1H, s, H-3'), 6.03 (1H, t, $^2J_{HF}$ = 56.2 Hz, H-8'), 6.17 (1H, d, *J* = 15.9 Hz, H-5), 7.79 (1H, d, *J* = 15.9 Hz, H-4); ^{13}C NMR (67.5 MHz, CD_3OD): δ 17.5 (C-9'), 19.3 (C-7'), 21.2 (C-6), 41.7 (C-5'), 78.4 (C-1'), 119.9 (t, J_{CF} = 244.2Hz, C-8'), 120.6 (C-2), 128.0 (C-3'), 129.9 (C-4), 136.7 (C-5), 150.2 (C-3), 165.2 (C-2'), 169.7 (C-1), 198.6 (C-4'); UV λ_{max} (MeOH) nm (ε): 249.5 (18,600); IR of the methyl ester ν_{max} ($CHCl_3$)

cm^{-1}: 3580, 3000, 2950, 1700, 1665, 1630, 1600, 1433, 1373, 1240, 1160, 1127, 1090, 1050; EIMS *m/z* (rel. int.): 300 $[M]^+$ (2), 282 (11), 241 (25), 231 (12), 213 (5), 203 (37), 190 (100), 175 (13), 162 (58), 147 (21), 134 (83), 119 (23), 111 (84); HR-EIMS: $[M]^+$ at *m/z* 300.1165 ($C_{15}H_{18}O_4F_2$ requires 300.1173). (±)-**42**. ^1H NMR (400 MHz, CD_3OD): δ 1.18 (3H, d, $^4J_{HF}$ = 1.0 Hz, H-8'), 1.92 (3H, d, *J* = 1.5 Hz, H-7'), 2.03 (3H, d, *J* = 1.0 Hz, H-6), 2.28 (1H, dd, *J* = 17.1 and 1.0 Hz, H-5'-*pro-R*), 2.79 (1H, d, *J* = 17.1 Hz, H-5'-*pro-S*), 5.76 (1H, s, H-2), 5.92 (1H, t, $^2J_{HF}$ = 56.2 Hz, H-9'), 5.95 (1H, s, H-3'), 6.23 (1H, dd, *J* = 15.9 Hz and $^4J_{HF}$ = 4.9 Hz, H-5), 7.79 (1H, d, *J* = 15.9 Hz, H-4); ^{13}C NMR (67.5 MHz, CD_3OD): δ 16.3 (C-8'), 19.1 (C-7'), 21.5 (C-6), 41.6 (C-5'), 50.6 (C-6') 78.8 (C-1'), 119.2 (t, J_{CF} = 244.1 Hz, C-9'), 120.4 (C-2), 127.6 (C-3'), 129.6 (C-4), 136.7 (C-5), 151.1 (C-3), 165.1 (C-2'), 169.8 (C-1), 198.6 (C-4'); UV λ_{max} (MeOH) nm (ε): 245.0 (20,200); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3580, 3000, 2940, 1700, 1665, 1630, 1600, 1433, 1373, 1238, 1160, 1120, 1085, 1050; EIMS *m/z* (rel. int.): 300 $[M]^+$ (1), 282 (6), 241 (19), 231 (9), 203 (54), 190 (100), 175 (13), 162 (54), 147 (18), 134 (77), 119 (22), 111 (93); HR-EIMS: $[M]^+$ at *m/z* 300.1163 ($C_{15}H_{18}O_4F_2$ requires 300.1173). (±)-**41**. ^1H NMR (400 MHz, CD_3OD): δ 1.08 (3H, s, H-9'), 1.91 (3H, d, *J* = 1.5 Hz, H-7'), 2.27 (3H, d, *J* = 1.2 Hz, H-6), 2.48 (1H, d, *J* = 17.7 Hz, H-5'-*pro-R*), 2.67 (1H, d, *J* = 17.7 Hz, H-5'-*pro-S*), 5.87 (1H, s, H-2), 5.93 (1H, br s, H-3'), 6.02 (1H, t, $^2J_{HF}$ = 56.5 Hz, H-8'), 6.20 (1H, d, *J* = 15.9 Hz, H-5), 6.49 (1H, d, *J* = 15.9 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 248.5 (19,700); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3590, 3000, 2950, 1710, 1667, 1630, 1613, 1433, 1358, 1256, 1162, 1090, 1055; EIMS *m/z* (rel. int.): 300 $[M]^+$ (3), 282 (11), 241 (25), 231 (12), 213 (4), 203 (17), 190 (100), 175 (11), 162 (55), 147 (20), 134 (83), 119 (21), 111 (50); HR-EIMS: $[M]^+$ at *m/z* 300.1171 ($C_{15}H_{18}O_4F_2$ requires 300.1173). (±)-**43**. ^1H NMR (400 MHz, CD_3OD): δ 1.17 (3H, s, H-8'), 1.89 (3H, d, *J* = 1.2 Hz, H-7'), 2.26 (3H, d, *J* = 1.2 Hz, H-6), 2.31 (1H, dd, *J* = 17.1 and 1.2 Hz, H-5'-*pro-R*), 2.83 (1H, d, *J* = 17.1 Hz, H-5'-*pro-S*), 5.85 (1H, s, H-2), 5.89 (1H, t, $^2J_{HF}$ = 55.9 Hz, H-9'), 5.94 (1H, qd, *J* = 1.2 and 1.2 Hz, H-3'), 6.26 (1H, dd, *J* = 15.6 Hz and $^4J_{HF}$ = 4.0 Hz, H-5), 6.50 (1H, d, *J* = 15.6 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 246.0 (21,300); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3590, 3000, 2950, 1710, 1670, 1632, 1613, 1433, 1358, 1255, 1162, 1120, 1085, 1053; EIMS *m/z* (rel. int.): 300 $[M]^+$ (4), 282 (10), 241 (27), 231 (12), 213 (4), 203 (17), 190 (100), 175 (11), 162 (55), 147 (20), 134 (83), 119 (21), 111 (50); HR-EIMS: $[M]^+$ at *m/z* 300.1178 ($C_{15}H_{18}O_4F_2$ requires 300.1173).

(±)-8',8',8'-*Trifluoro-ABA* (**3**), *its* (2E)-*isomer* (**46**), 9',9',9'-*trifluoro-ABA* (**47**), *and its* (2E)-*isomer* (**48**)

In the same manner as for **39**, (±)-(2*Z*,4*E* and 2*E*,4*E*)-methyl 5-(6'-difluoromethyl-2',6'-dimethyl-1'-cyclohexen-1'-yl)-3-methyl-2,4-pentadienoate (215 mg, **44**) gave didehydro compound **45** (75 mg) as a crude oil. In the same manner as for **40**, the didehydro compound **45** (74 mg) gave 0.5 mg of (±)-**3** as an amorphous powder, 1.1 mg of (±)-**46** as an oil, 2.1 mg of (±)-**47** as an oil, and 3.4 mg of (±)-**48** as an oil. (±)-**3**. ^1H NMR (400 MHz, CD_3OD): δ 1.26 (3H, s, H-9'), 1.95 (6H, d, *J* = 1.2 Hz, H-6 and H-7'), 2.67 (1H, d, *J* = 19.6 Hz, H-5'-*pro-R*), 2.72 (1H, d, *J* = 19.6 Hz, H-5'-*pro-S*), 5.85 (1H, s, H-2), 5.92 (1H, br s, H-3'), 5.98 (1H, d, *J* = 15.9 Hz, H-5), 7.69 (1H, d, *J* = 15.9 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD): δ 19.1 (C-9'), 20.1 (C-7'), 21.4 (C-6), 45.1 (C-5'), 79.2 (C-1'), 118.5 (C-2), 128.2 (C-

3'), 129.1 (C-4), 130.3 (q, J_{CF} = 276.3Hz, C-8'), 133.1 (C-5), 167.3 (C-2'), 169.8 (C-1), 198.2 (C-4'); UV λ_{max} (MeOH) nm (ε): 242.5 (22,800); EIMS of the methyl ester *m/z* (rel. int.): 332 $[M]^+$ (3), 314 (4), 301 (6), 300 (5), 272 (3), 259 (7), 203 (2), 190 (35), 162 (16), 149 (6), 134 (26), 125 (100), 112 (26); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 332.1221 ($C_{16}H_{19}O_4F_3$ requires 332.1236). (±)-**47**. ^1H NMR (400 MHz, CD_3OD): δ 1.33 (3H, s, H-8'), 1.90 (3H, s, H-7'), 1.94 (3H, d, *J* = 1.2 Hz, H-6), 2.38 (1H, dd, *J* = 16.8 and 0.9 Hz, H-5'-*pro-R*), 2.91 (1H, d, *J* = 16.8 Hz, H-5'-*pro-S*), 5.86 (1H, s, H-2), 5.94 (1H, br s, H-3'), 6.02 (1H, dq, *J* = 16.2 Hz and $^4J_{HF}$ = 2.8 Hz, H-5), 7.58 (1H, d, *J* = 16.2 Hz, H-4); ^{13}C NMR (67.5 MHz, CD_3OD): δ 16.5 (C-8'), 19.1 (C-7'), 21.0 (C-6), 42.8 (C-5'), 52.2 (C-6'), 78.5 (C-1'), 122.0 (C-2), 127.2 (C-3'), 129.1 (q, J_{CF} = 284.0 Hz, C-9'), 129.5 (C-4), 135.0 (C-5), 148.7 (C-3), 164.9 (C-2'), 196.7 (C-4'); UV λ_{max} (MeOH) nm (ε): 239.5 (24,400); EIMS of the methyl ester *m/z* (rel. int.): 332 $[M]^+$ (6), 314 (4), 301 (13), 300 (17), 273 (6), 272 (6), 259 (20), 203 (4), 190 (67), 162 (48), 149 (17), 134 (52), 125 (100), 112 (17); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 332.1208 ($C_{16}H_{19}O_4F_3$ requires 332.1236). (±)-**46**. ^1H NMR (400 MHz, CD_3OD): δ 1.24 (3H, s, H-9'), 1.92 (3H, d, *J* = 1.2 Hz, H-7'), 2.23 (3H, s, H-6), 2.73 (2H, s, H-5'), 5.90 (1H, s, H-2), 5.94 (1H, br s, H-3'), 6.17 (1H, d, *J* = 15.6 Hz, H-5), 6.53 (1H, d, *J* = 15.6 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 242.5 (23,200); EIMS of the methyl ester *m/z* (rel. int.): 332 $[M]^+$ (6), 314 (4), 301 (13), 300 (13), 273 (6), 272 (6), 259 (20), 203 (5), 190 (75), 162 (35), 149 (10), 134 (51), 125 (100), 112 (21); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 332.1197 ($C_{16}H_{19}O_4F_3$ requires 332.1236). (±)-**48**. ^1H NMR (400 MHz, CD_3OD): δ 1.34 (3H, s, H-8'), 1.92 (3H, d, *J* = 1.2 Hz, H-7'), 2.25 (3H, d, *J* = 1.2 Hz, H-6), 2.44 (1H, dd, *J* = 17.1 and 1.2 Hz, H-5'-*pro-R*), 2.94 (1H, d, *J* = 17.1 Hz, H-5'-*pro-S*), 5.86 (1H, s, H-2), 5.97 (1H, dq, *J* = 1.2 and 1.2 Hz, H-3'), 6.31 (1H, dq, *J* = 15.6 Hz and $^4J_{HF}$ = 1.4 Hz, H-5), 6.49 (1H, d, *J* = 15.6 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 241.5 (21,800); EIMS of the methyl ester *m/z* (rel. int.): 332 $[M]^+$ (6), 314 (4), 301 (14), 300 (14), 273 (6), 272 (5), 259 (20), 203 (4), 190 (70), 162 (35), 149 (14), 134 (52), 125 (100), 112 (21); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 332.1180 ($C_{16}H_{19}O_4F_3$ requires 332.1236).

Optical resolution of (±)-**2**, **-42**, **-3**, **-47** *and -ABA*

Racemic mixtures of **2** and **42** were separated into enantiomers by HPLC on Chiralpak OD (250 x 4.6 mm, Daicel; solvent, 13% isopropanol in hexane containing 0.1% TFA; flow rate, 0.7 ml min^{-1}; detection, 254 nm). The materials at t_R 10.6 and 17.2 min of **2** (26 mg) were collected to give (+)- and (-)-**2** (12.3 and 13.2 mg) with optical purity of 99.6%, and the materials at t_R 9.4 and 17.4 min of **42** (57 mg) were collected to give (+)- and (-)-**42** (28.0 and 28.2 mg) with optical purity of 99.9 and 99.8%, respectively. (+)-**2**: $[\alpha]_D^{27}$ +236.6° (MeOH; *c* 0.410); CD λ_{ext} (MeOH) nm (Δε): 229 (-11.7), 263 (+13.0), 320 (-1.5). (-)-**2**: $[\alpha]_D^{27}$ -240.9° (MeOH; *c* 0.440); CD λ_{ext} (MeOH) nm (Δε): 225 (+12.2), 255 (-11.9), 317 (+1.6). (+)-**42**: $[\alpha]_D^{27}$ +336.4° (MeOH; *c* 0.933); CD λ_{ext} (MeOH) nm (Δε): 226 (-15.8), 262 (+17.4), 319 (-2.3). (-)-**42**: $[\alpha]_D^{27}$ -343.6° (MeOH; *c* 0.940); CD λ_{ext} (MeOH) nm (Δε): 225 (+16.0), 258 (-16.7), 317 (+2.2). Racemic mixtures of **3** and **47** were separated into enantiomers by HPLC on Chiralpak OD (solvent, 11% and 8% isopropanol, respectively, in hexane containing 0.1% TFA; flow rate, 1.0 ml min^{-1}; detection, 254 nm). The materials at t_R 8.0 and 15.7 min of **3** (0.5 mg) were collected to give (+)- and (-)-**3** (0.2 and 0.2 mg) with optical purity of 99.8 and 99.7%, respectively, and

the materials at t_R 11.1 and 16.3 min of **47** (2 mg) were collected to give (+)- and (-)-**47** (0.9 and 0.9 mg) with optical purity of 99.6 and 99.7%, respectively. (+)-**3**: $[\alpha]_D^{17}$ +283.3° (MeOH; *c* 0.012); CD λ_{ext} (MeOH) nm (Δε): 227 (-17.5), 257 (+18.9), 317 (-1.7). (-)-**3**: $[\alpha]_D^{17}$ -290.0° (MeOH; *c* 0.020); CD λ_{ext} (MeOH) nm (Δε): 228 (+15.7), 259 (-18.1), 321 (+2.5). (+)-**47**: $[\alpha]_D^{17}$ +390.6° (MeOH; *c* 0.064); CD λ_{ext} (MeOH) nm (Δε): 229 (-19.9), 258 (+22.3), 315 (-2.1). (-)-**47**: $[\alpha]_D^{17}$ -391.5° (MeOH; *c* 0.071); CDλ_{ext} (MeOH) nm (Δε): 227 (+20.9), 256 (-20.4), 322 (+2.3). A commercially available racemic mixture of ABA was separated into its enantiomers by HPLC on a Chiralcel OD (solvent, 15% isopropanol in hexane containing 0.1% TFA; flow rate, 0.5 ml min^{-1}; detection, 254 nm). The materials at t_R 11.4 and 16.0 min of (±)-ABA (10 mg) were collected to give (+)- and (-)-ABA (4.7 and 4.6 mg) with optical purity of 99.9 and 99.6%, respectively. (+)-ABA: $[\alpha]_D^{26}$ +492.9° (*c* 0.071, $CHCl_3$) and +444.9° (*c* 0.071, MeOH); CD λ_{ext} (MeOH) nm (Δε): 227 (-34.9), 260 (+42.7), 320 (-2.5); CD λ_{ext} ($CHCl_3$) nm (Δε): 231 (-23.4), 265 (+38.7), 323 (-2.4). (-)-ABA: $[\alpha]_D^{26}$ -481.3° (*c* 0.141, $CHCl_3$) and -442.2° (*c* 0.141, MeOH); CD λ_{ext} (MeOH) nm (Δε): 229 (+34.1), 259 (-41.1), 323 (+2.2); CD λ_{ext} (MeOH) nm (Δε): 228 (+27.5), 263 (-36.2), 321 (+3.7).

(-)-PA

(-)-PA was prepared by hydrolysis of the β-hydroxy-β-methylglutaryl ester of 8'-hydroxyabscisic acid.[37]

Lettuce germination assay[78]

Fifty seeds of lettuce (*Lactuca sativa* L. cv. Grand Rapids) were placed on two sheets of Toyo No. 2 filter paper (5.5 cm in diameter) soaked in 3 ml of a test solution and allowed to germinate under illumination (3000 lux) at 25°C. After 48 hr, the inhibition ratio was calculated. The inhibition ratio is defined as $[(A - B)/A] \times 100$, where A = the number (47) of seeds that germinated when water was used, and B = the number of seeds that germinated when a test compound was used. All tests were conducted at least twice.

Rice elongation assay[79]

Seeds of rice (*Oryza sativa* L. cv. Nihonbare) were soaked in EtOH for 5 min, sterilized with 1% antiformin ($NaClO_4$) for 1 hr, and washed with running tap water for 3 hr. The sterilized seeds were allowed to germinate in water for two days at 30°C. The resulting seedlings were placed in a glass tube containing 2 ml of a test solution, and grown with the tube sealed by a sheet of polyethylene film under continuous illumination at 30°C. The length of the second leaf sheath was measured after seven days, and the inhibition ratio was calculated. The inhibition ratio is defined as $[(A - B)/A] \times 100$, where A = the mean length (28 mm) of the second leaf sheath when water was used, and B = the mean length of the second leaf sheath when a test compound was used. All tests were conducted at least twice.

α-Amylase assay[80]

Barley (*Hordeum vulgare* L. cv. Himalaya) seeds were sterilized in 4% antiformin for 3 hr and then rinsed with sterilized distilled water. The seeds were next soaked in sterilized distilled water at 5°C for 20

hr and cut in half. Two of the halves without embryos were placed in a 30-ml screw-capped vial containing 1 ml of a test solution. Each vial contained streptomycin (0.5 mg), GA_3 (10^{-7} M), the appropriate amount of a test compound, and water to a total volume of 1 ml. The sealed vials were placed in an incubator at 30°C for 48 hr in the dark. Distilled water (9 ml) was then added to each vial, and 50 ml of the resulting solution was assayed for its absorbance at 660 nm by the Somogyi-Nelson method[94] to calculate the inhibition ratio. The inhibition ratio is defined as $[(A - C)/(A - B)] \times 100$, where A = the absorbance (1.1) when 10^{-7} M GA_3 was used, B = the absorbance (0.07) when water was used, and C = the absorbance when a test compound was used. All tests were conducted at least twice.

Stomata assay[81]

Fully expanded leaves of spiderwort (*Tradescantia reflexa* Rafin) were cut and floated on water in the dark for 5 hr before use to ensure that the stomata were closed at the beginning of incubation. Strips of the epidermis about 5 mm square were detached from the abaxial leaf surfaces under dim light. To 3.9 ml of a 10^{-2} M citrate buffer (pH 5.5) in a 10-ml vial, a test compound in 100 ml of MeOH was added. Three strips were floated on the solution and incubated at 25°C. Illumination was provided by a tungsten lamp (500 W) with a light intensity of approximately 12 klux. After 3 hr, the widths of the apertures of 10 stomata on each strip were measured under a microscope. The stomatal aperture ratio is defined as $(B / A) \times 100$, where A = the mean aperture (9.6 mm) when the buffer was used, and B = the mean aperture when a test compound was used. All tests were conducted at least twice.

I.3 8'-Methoxyabscisic Acid as the Cyclization-Resistant Analogue

Design Concept

As described in Chapter I.1, an approach that confers resistance to the cyclization of 8'-HOABA to PA is lowering the nucleophilicity of the 8'-oxygen. The simplest way is to protect the 8'-hydroxyl group with a chemically stable moiety. Esterification is not suitable because the conjugate **20** of 8'-HOABA had low activity due to easy decomposition to inactive PA by hydrolysis.[37] The author selected the methyl etherification of the 8'-hydroxyl group and designed 8'-methoxy-ABA (**4**) as the first analogue resistant to the cyclization.

Results and Discussion

Synthesis and optical resolution

8'-Methoxy-ABA (**4**) were synthesized along with 9'-methoxy-ABA (**62**) by a modification of the method reported for the synthesis of (±)-methyl phaseate[73] (Fig. 8). After acetylating the hydroxymethyl ketone **30** to give **49**, the carbonyl group of **49** was protected, and subsequent hydrolysis gave hydroxymethyl ketal **50**. Methylation of **50** gave methoxymethyl ketal **51**, which was then acid-treated to give methoxymethyl ketone **52**. The reaction of **52** with alkynyl lithium gave THP ether **53**. Deprotec-

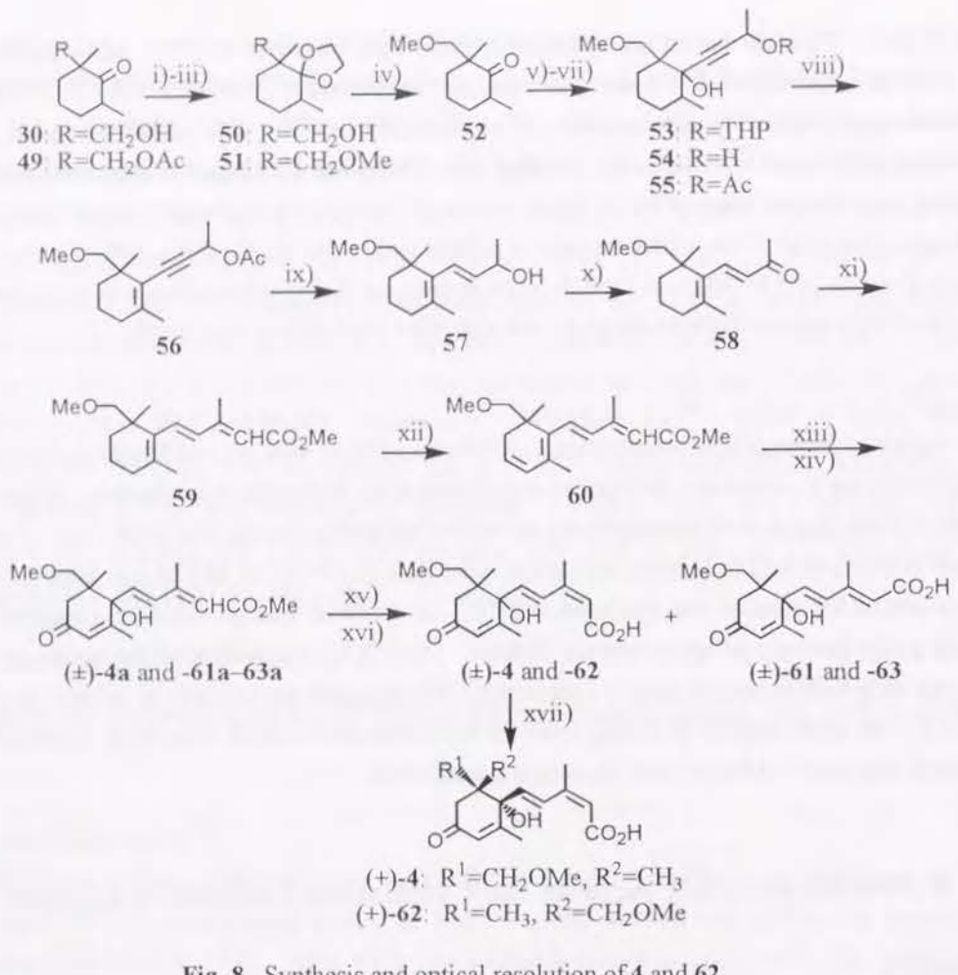


Fig. 8. Synthesis and optical resolution of **4** and **62**.

i) Ac_2O, pyridine ii) a) ethylene glycol, *p*-TsOH b) NaOH iii) NaH, MeI iv) HCl v) Li≡⟨OTHP vi) pyridinium *p*-TsOH vii) Ac_2O, pyridine viii) $POCl_3$, pyridine ix) Red-Al x) MnO_2 xi) $Ph_3P{=}CHCO_2Me$ xii) a) NBS, BPO b) quinoline xiii) rose bengal, O_2, hν xiv) basic alumina xv) NaOH xvi) ODS HPLC xvii) HPLC with Chiralcel OD.

tion of **53** gave acetylenic diol **54**, which was then acetylated to afford acetylenic acetate **55**. Dehydration of **55** with phosphorus oxychloride in pyridine gave enyne acetate **56** in a low yield (16%). The recovered **55** after this step was dehydrated again with thionyl chloride in pyridine to afford **56** in a 58% yield. Reduction of **56** gave dienol **57**, which was oxidized to give dienone **58**. The Wittig reaction of **58** gave methyl ester **59** as a mixture of (2*Z*)- and (2*E*)-isomers. Bromination of **59** and then dehydrobromination gave dehydro compound **60**. Photosensitized oxygenation of **60** and subsequent treatment with basic alumina gave the methyl esters of methoxy-ABAs (Me methoxy-ABAs): four stereoisomers resulting from the 2*Z*/2*E* isomerism and from the *cis* or *trans* relationship of the 6'-methoxymethyl group to the 1'-hydroxyl group (*cis*:*trans* = about 1:3, measured by HPLC of the free

acids). This ratio of 1:3 resulted from the stereoselective addition of a singlet oxygen from the less-hindered site on the opposite side of the 6'-methoxymethyl group. Hydrolysis of the methyl esters gave an isomeric mixture of (±)-8'-methoxy-ABA (**4**) and its (±)-(2*E*)-isomer (**61**), and of (±)-9'-methoxy-ABA (**62**) and its (±)-(2*E*)-isomer (**63**) (about 1:2:3:6, measured by HPLC). This mixture was separated into its components by HPLC with an ODS column.

Compounds (±)-**4**, -**61**, -**62** and -**63** were identified by ^{1}H NMR spectral data of corresponding methyl esters (±)-**4a**, -**61a**, -**62a** and -**63a**, respectively. The chemical shift of the 4-proton led to assigning the configuration of 2*Z* to (±)-**4a** (δ 7.87) and (±)-**62a** (δ 7.93), and the configuration of 2*E* to (±)-**61a** (δ 6.52) and (±)-**63a** (δ 6.56). Table 3(a) shows ^{1}H NMR spectral data in $CDCl_3$ for (±)-**4a** and (±)-**62a**. The 6'-methyl group of (±)-**4a** (δ 1.15) appeared in a higher field than that of (±)-**62a** (δ 1.25). The 9'-protons of the methyl ester of (±)-ABA (**1a**) appear in a higher field than the 8'-protons,[76] so the spectrum of (±)-**4a** lacked the signal arising from the 8'-protons of (±)-**1a**, and that of (±)-**74a** lacked the signal arising from the 9'-protons. These findings suggested that (±)-**4a** was the methyl ester of (±)-8'-methoxy-ABA and that (±)-**62a** was the methyl ester of (±)-9'-methoxy-ABA. The chemical shifts of the methylene protons of the methoxymethyl group, the 1'-hydroxyl group and the C-5 proton were evidence for this conclusion. The methylene protons of (±)-**4a** appeared in fields lower than the fields of (±)-**62a**. This downfield shift was interpreted as due to the deshielding effect of the 4'- carbonyl group, which was closer to C-8' than to C-9'. The 1'-hydroxyl group of (±)-**4a** appeared in a lower field than that of (±)-**62a**. This downfield shift was attributed to hydrogen bonding with the oxygen of the 8'-methoxyl group, which is *cis* to the hydroxyl group. The 5-proton of (±)-**62a** was in a lower field than that of (±)-**4a**, and this finding suggested that the 5-proton of (±)-**62a** was deshielded by the oxygen of the 9'-methoxyl group, which is *cis* to the 5-proton. These results showed that (±)-**4a** was the methyl ester of (±)-8'-methoxy-ABA, and that (±)-**62a** was the methyl ester of (±)-9'-methoxy-ABA; (±)-**4** was (±)-8'-methoxy-ABA and (±)-**62** was (±)-9'-methoxy-ABA. The relative configurations at C-6' of the (2*E*)-isomers were identified in the same way as that for the (2*Z*)-isomers: (±)-**61** was (±)-(2*E*)-8'-methoxy-ABA, and (±)-**63** was (±)-(2*E*)-9'-methoxy-ABA.

(±)-**4** and -**62** were optically resolved by HPLC in a Chiralpak AD column to afford the (+)- and (-)-enantiomers with an optical purity of more than 99%. The CD spectra of the (+)-enantiomers showed the same positive first and negative second Cotton effects as those of (+)-ABA.[77] Therefore, the absolute configuration at C-1' of both (+)-**4** and -**62** is *R*, while that at C-6'is *S* for (+)-**4** and *R* for (+)-**62**.

Biological activity

The optically active analogs were compared with the (+)- and (-)-enantiomers of ABA for inhibitory activity by means of the four bioassays described in Chapter I.2. The IC_{50} values are summarized in Table 4. The (±)-(2*E*)-isomers were inactive (data not shown).

The activity of (+)-**4** was 4.5 times higher than that of (+)-ABA in the rice assay in terms of IC_{50} values, whereas it was similar in the lettuce and α-amylase assays and weaker in the stomata assay. The effect of (+)-**4** was longer lasting in the rice, than in the lettuce assay (Fig. 9). These are analogous to the activities of (+)-**2** and -**3**, so the highly inhibitory effect of (+)-**4** on the elongation of the rice seedlings was also considered to result from delayed metabolism. On the other hand, the IC_{50} value of (+)-**62** was 0.4

Table 3. ^{1}H NMR Data for (±)-**1a**, -**4a** and -**62a**

(a) $CDCl_3$

H	(±)-**1a**[76]	(±)-**4a**	(±)-**62a**
2	5.753 m (0.44, 0.26)	5.73 br s	5.75 br s
4	7.871 dd (15.36, 0.26)	7.87 dd (15.9, 0.9)	7.93 d (15.9)
5	6.152 dd (15.36, 0.23)	6.01 d (15.9)	6.15 d (15.9)
6	2.009 d (0.44)	2.00 d (1.2)	2.01 d (1.2)
3'	5.942 m (1.46, 0.37)	5.96 qd (1.2, 1.2)	5.89 qd (1.2, 1.2)
5'-*proR*	2.288 dd (15.71, 0.37)	2.22 dd (17.7, 1.2)	2.08 dd (17.1, 1.2)
5'-*proS*	2.478 dd (15.71, 0.27)	2.45 d (17.7)	2.29 d (17.1)
7'	1.923 d (0.47)	1.97 d (1.2)	1.90 d (1.2)
8'	1.110 d (0.27)	3.24 d (9.5)	1.25 br s
		3.80 d (9.5)	
9'	1.014 s	1.15 s	3.10 d (9.2)
			3.45 d (9.2)
1'-OH	—	4.97 s	4.02 s
OMe	—	3.35 s	3.33 s
CO_2Me	3.706 s	3.70 s	3.71 s

(b) CD_3OD

H	(±)-**1a**	(±)-**4a**	(±)-**62a**
2	5.76 br s	5.77 br s	5.76 br s
4	7.79 dd (16.2, 0.9)	7.79 dd (16.2, 0.9)	7.86 dd (15.9, 0.9)
5	6.27 dd (16.2, 0.6)	6.22 dd (16.2, 0.6)	6.32 dd (15.9, 0.6)
6	2.04 d (1.2)	2.04 d (1.2)	2.04 d (1.2)
3'	5.94 qd (1.2, 1.2)	5.95 qd (1.2, 1.2)	5.90 qd (1.2, 1.2)
5'-*proR*	2.19 dd (17.1, 1.2)	2.49 dd (17.1, 1.2)	2.16 dd (17.1, 1.2)
5'-*proS*	2.53 d (17.1)	2.37 d (17.1)	2.60 d (17.1)
7'	1.93 d (1.2)	1.93 d (1.2)	1.90 d (1.2)
8'	1.07 s	3.44 s	1.13 br d (0.9)
9'	1.02 s	1.06 s	3.29 dd (9.2, 1.8)
			3.36 d (9.2)
OMe	—	3.29 s	3.29 s
CO_2Me	3.66 s	3.71 s	3.67 s

Values for the chemical shifts are in δ (ppm), and those for the coupling constants in parentheses are in *J* (Hz). Abbreviations: s, singlet; d, doublet; q, quartet; br, broad.

μM in the lettuce germination assay, in which its activity was 7 times higher and longer-lasting than that of (+)-ABA (IC_{50} = 2.8 μM) (Fig. 9). In the other assays, (+)-**62** had the slightly stronger or weaker activity than (+)-ABA. As described in Chapter I.2, conjugation is the major metabolic pathway in lettuce

Table 4. The IC_{50} Values for Optically Active ABA, **4** and **62** in Four Bioassays

Compound	IC_{50} in assay			
	Rice seedling elongation (μM)	Lettuce seed germination (μM)	Barley α-amylase induction (μM)	Spiderwort stomatal opening (nM)
(+)-ABA	3.2	2.8	3.5	2.9
(+)-**4**	0.7	3.3	4.8	18
(+)-**62**	3.4	0.4	1.4	4.8
(-)-ABA	4.3	7.4	7.0	20
(-)-**4**	265	30	>100	25
(-)-**62**	39	14	31	20

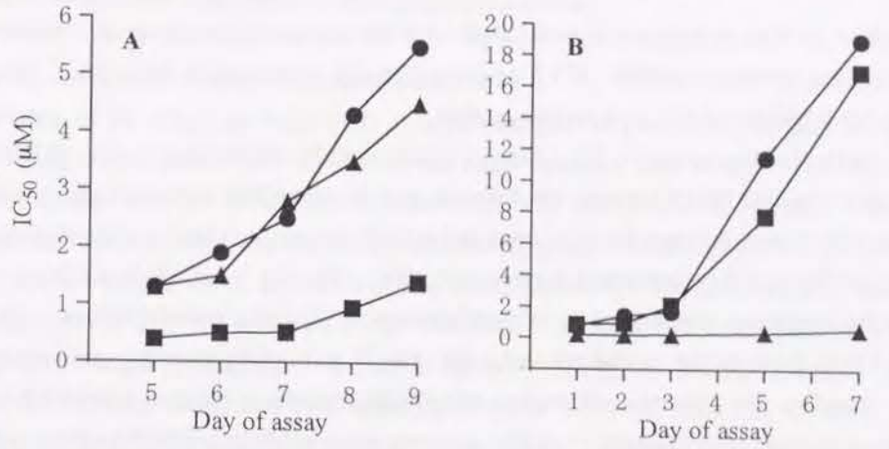


Fig. 9. Changes with time in the inhibitory activity of (+)-**1**, -**4**, and -**62** in the rice seedlings elongation (A) and the lettuce seed germination (B).
●, (+)-**1**; ■, (+)-**4**; ▲, (+)-**62**.

seeds . Therefore, one explanation of the high and long-lasting activity of (+)-**62** in the lettuce assay may be the high resistance to the conjugation that results from a steric or electronic effect of the 9'-methoxy group. Another may be the high affinity for the active site on the receptor in lettuce seeds owing to the 9'-methoxy group or the conformation of the molecule.

The activity of the (-)-enantiomer of **4** and **62** was weak in all the assays; it was 1.0 to 10% that of (+)-ABA and weaker than (-)-ABA. This is similar to the activity of (-)-**2** and -**3**, so the decreased activities of (-)-**4** and -**62** can be explained by the steric or electronic effect of the 8'- and 9'-methoxy group corresponding to the C-2' side of (+)-ABA, where the steric requirement for activity is strict,[93] as are those of (-)-**2** and-**3**.

Factors affecting activity of (+)-62

As described above, conformation may explain high activity of (+)-**62** in the lettuce assay. To verify this notion, the favored conformation of **62** along with **4** in solution was examined by NMR. The

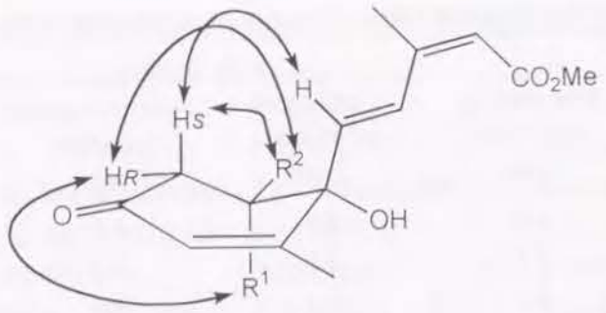


Fig. 10. Favored conformations of **1a**, **4a** and **62a**.
1a: R^1=R^2=Me; **4a**: R^1=CH_2OMe, R^2=Me; **62a**: R^1=Me, R^2=CH_2OMe.
Arrows represent the observed NOEs.

favored conformation of ABA in solution is a half-chair with the side chain pseudo-axial. However, the active conformation has not been clarified. If **62** adopts a different conformation from ABA, then it may be the active state for inhibition of lettuce seed germination.

In the nuclear Overhauser effect (NOE) difference spectra and by NOE spectroscopy (NOESY) in a $CDCl_3$ solution, (±)-**62a** had NOEs between the 5-proton and the downfield 5'-proton (the *pro-S* proton which is *cis* to the side chain), between the 9'-protons and both 5'-protons and between the 8'-protons and the upfield 5'-proton (the *pro-R* proton which is *cis* to C-8') (Fig. 10). In ^{1}H NMR in a $CDCl_3$ solution, (±)-**62a** showed the long-range coupling of the W-type between the 3'- and 5'-*pro-R* protons. These data completely agreed with those of the methyl ester of ABA (**1a**),[76] and (±)-**4a** gave the same result. In a $CDCl_3$ solution, therefore, the favored conformation of (±)-**62a** and -**4a** is equal to that of ABA; it is a half-chair with the side chain pseudo-axial. (±)-**62a** gave the same result in a CD_3OD solution, whereas (±)-**4a** afforded a strange result. In CD_3OD, (±)-**62a** and -**1a** showed W-type coupling with the 3'-proton in the upfield proton of the 5'-protons, whereas (±)-**4a** showed it in the downfield proton. The 5'-proton possessing the NOE with the 5-proton was the downfield proton for (±)-**1a** and -**62a**, and the upfield proton for (±)-**4a**. This means that chemical shifts of the 5'-protons of (±)-**4a** are inverted despite favoring the same conformation as (±)-**1a** and -**62a**. It is unclear what effect causes such a inversion of the chemical shifts of 5'-protons. These results suggest that the high effect of (+)-**62** cannot be attributed to its conformation.

Thus (+)-**62** may have high resistance to the conjugation or high affinity for the receptor which results from a steric or electronic effect of the 9'-methoxy group itself. To examine which of the steric and electronic effects affects the activity, (+)-9'-methyl- and ethyl-ABAs were tested in the lettuce assay.[95] Both showed longer-lasting activity compared with (+)-ABA, but less that of (+)-**62**, so the high activity of (+)-**62** may be caused by both the steric and electronic effects of the methoxyl group.

In conclusion, methyl etherification of the 8'-hydroxyl group of 8'-HOABA caused high and long-lasting activity as designed, probably owing to the delayed metabolism caused by resistance to cyclization to PA or blockage of hydroxylation at C-8' to give a hemiacetal, which can convert into aldehyde or PA-like acetal. However, its effect was smaller than that in the di- and trifluorinations. The cytochrome P-

450 enzyme can catalyze the oxidative dealkylation of the ether,[60] so (+)-**4** may be inactivated via demethylation of the 8'-methoxy group, as well as conjugation to ABA-GE.

Experimental

General procedures

^{1}H NMR spectra were recorded with TMS as the internal standard by Hitachi R-22 (90 Mz) and Jeol GX400 (400 Mz) apparatus, using $CDCl_3$ or CD_3OD as the solvent. For clarity, the conventional ABA numbering system is used in the assignment of peaks in the ^{1}H NMR spectra. NOESY was performed on Jeol GSX270J (270 Mz) apparatus, and mass spectra were obtained with a Jeol JMS-DX300/DA5000 mass spectrometer. GC-MS was conducted with a 1% OV-17 column (2.6 mm x 1 m) in the EI mode. CD spectra were recorded with a Jasco J-600 spectropolarimeter.

(±)-2-*Acetoxymethyl*-2,6-*dimethyl*-1-*cyclohexanone* (**49**)

A mixture of **30** (63 g) and Ac_2O (130 g) in pyridine (120 ml) was stirred at room temperature for 21 hr. The mixture was poured into ice-cooled H_2O and extracted with ether. The organic layer was successively washed with 0.1 N HCl and saturated $NaHCO_3$, and dried over Na_2SO_4. The solvent was evaporated, and the residual oil was chromatographed on silica gel with hexane-EtOAc (9:1) as the eluent to afford 76 g (95% yield) of **49** as a mixture of two diastereomers (52:48, determined by integrating the 2-methyl singlets in the ^{1}H NMR spectrum). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01 (3H, d, J = 6.4 Hz, Me-6), 1.02 (3H, d, J = 6.4 Hz, Me-6), 1.10 (3H, s, Me-2), 1.21 (3H, s, Me-2), 1.34-2.10 (12H, m, H-3, H-4, H-5), 2.03 (3H, s, OAc), 2.05 (3H, s, OAc), 2.66 (1H, m, H-6), 2.66 (1H, m, H-6), 4.02 (1H, d, J = 11.0 Hz, OCH_2), 4.03 (1H, d, J = 11.0 Hz, OCH_2), 4.17 (1H, d, J = 11.0 Hz, OCH_2), 4.50 (1H, d, J = 11.0 Hz, OCH_2); GC-MS *m/z* (rel. int.): 198 $[M]^+$ (11), 155 (6), 138 (35), 124 (28), 110 (72), 95 (73), 81 (57), 68 (100).

(±)-2-*Hydroxymethyl*-2,6-*dimethyl*-1-*cyclohexanone ethylene ketal* (**50**)

A mixture of **49** (71 g), ethylene glycol (26.7 g) and *p*-toluenesulfonic acid monohydrate (1.1 g) in benzene (200 ml) was refluxed for 6 hr, the H_2O produced being separated by a Soxhlet extractor packed with Drierite. The reaction mixture was washed with saturated $NaHCO_3$ and extracted with ether. The organic layer was washed with H_2O and evaporated to afford a crude residual oil (79 g). To a solution of this crude product in MeOH (200 ml) was added 1 N NaOH (400 ml). The mixture was stirred at room temperature for 1 hr, after which it was diluted with H_2O (400 ml) and extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4 and then evaporated. Chromatography of the residual oil on silica gel with hexane-EtOAc (17:3-7:3) afforded 27.6 g (39% yield) of **50** as a mixture of two diastereomers (about 1:1, determined by TLC analysis). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, d, J = 6.4 Hz, Me-6), 0.97 (3H, s, Me-2), 0.99 (3H, d, J = 6.4 Hz, Me-6) 1.20 (3H, s, Me-2), 1.28-1.62 (12H, m, H-3, H-4, H-5), 2.07 (2H, m, H-6), 3.27 (1H, d, J = 11.3 Hz, OCH_2), 3.43 (1H, d, J = 11.6 Hz, OCH_2), 3.50 (1H, d, J = 11.6 Hz, OCH_2), 4.05 (8H, m, OCH_2CH_2O), 4.13 (1H, d, J = 11.3 Hz, OCH_2); GC-MS *m/z* (rel. int.): 200 $[M]^+$ (8), 183 (2), 169 (10), 130 (5), 113 (100), 100 (9), 69 (12).

(±)-2-*Methoxymethyl*-2,6-*dimethyl*-1-*cyclohexanone ethylene ketal* (**51**)

To a stirred solution of **50** (50.9 g) and methyl iodide (184.6 g) in THF (300 ml) was added NaH (60% in oil, 35 g) in THF (150 ml). The mixture was stirred at room temperature for 16 hr, and after being quenched with H_2O, the mixture was extracted with ether. The organic layer were washed with H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (9:1) to afford 50 g (92% yield) of **51** as a mixture of two diastereomers (59:41, determined by integrating the 2-methyl singlets in the ^1H NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$): δ 0.81 (3H, d, J = 6.4 Hz, Me-6), 0.81 (3H, d, J = 6.7 Hz, Me-6), 0.88 (3H, s, Me-2), 1.07 (3H, s, Me-2), 1.29-1.74 (12H, m, H-3, H-4, H-5), 1.90 (1H, m, H-6), 1.99 (1H, m, H-6), 3.14 (1H, d, J = 8.2 Hz, OCH_2), 3.23 (1H, d, J = 8.2 Hz, OCH_2), 3.24 (1H, d, J = 9.0 Hz, OCH_2), 3.34 (3H, s, OMe), 3.62 (1H, d, J = 9.0 Hz, OCH_2), 4.03 (4H, m, OCH_2CH_2O), 4.03 (4H, m, OCH_2CH_2O); GC-MS *m/z* (rel. int.): 214 $[M]^+$ (10), 199 (4), 183 (7), 169 (12), 143 (2), 127 (4), 113 (100), 100 (9), 87 (3), 69 (10).

(±)-2-*Methoxymethyl*-2,6-*dimethyl*-1-*cyclohexanone* (**52**)

A mixture of **51** (49.8 g) and 2% methanolic HCl (500 ml) was stirred at room temperature for 39 hr and then extracted with ether. The organic layer was successively washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (9:1-4:1) to afford 38.4 g (97% yield) of **52** as a mixture of two diastereomers (52:48, determined by integrating the 2-methyl singlets in the ^1H NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$): δ 1.00 (3H, d, J = 6.7 Hz, Me-6), 1.02 (3H, d, J = 6.4 Hz, Me-6), 1.08 (3H, s, Me-2), 1.17 (3H, s, Me-2), 1.33-2.07 (12H, m, H-3, H-4, H-5), 2.62 (1H, m, H-6), 2.63 (1H, m, H-6), 3.26 (1H, d, J = 9.2 Hz, OCH_2), 3.30 (3H, s, OMe), 3.31 (1H, d, J = 8.9 Hz, OCH_2), 3.35 (3H, s, OMe), 3.47 (1H, d, J = 9.2 Hz, OCH_2), 3.68 (1H, d, J = 8.9 Hz, OCH_2); GC-MS *m/z* (rel. int.): 170 $[M]^+$ (6), 155 (3), 138 (35), 125 (8), 110 (16), 101 (13), 95 (21), 88 (100), 83 (45), 68 (23), 54 (17).

(±)-4-(1'-*Hydroxy*-2'-*methoxymethyl*-2',6'-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*-*THP ether* (**53**)

To a stirred solution of 1-methyl-2-propynyl THP ether (77 g) in THF (100 ml) was added dropwise *n*-BuLi (a 1.6 M solution in hexane, 310 ml) during 50 min at -75°C under nitrogen. After being stirred for 1 hr, the reaction mixture was warmed to -25°C, and **52** (38.4 g) in THF (100 ml) was added dropwise to the stirred mixture. The mixture was stirred for 2 hr at -25 to -10°C and then warmed to room temperature. After being quenched with 0.1 M NH_4Cl (300 ml), the mixture was extracted with ether, and the organic layer was successively washed with 0.1 M NH_4Cl and H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (4:1) to afford 43.1 g (59% yield) of **53** as a mixture of diastereomers. A portion of the mixture was chromatographed on silica gel with hexane-EtOAc (40:1-9:1) to obtain one major diastereomer. ^1H NMR: (90 MHz, $CDCl_3$): δ 1.04 (3H, d, J = 6.1 Hz, Me-6), 1.23 (3H, s, Me-2), 1.46 (3H, d, J = 6.0 Hz, H-1), 1.27-2.03 (15H, m, H-3', H-4', H-5', H-6', H-2", H-3", H-4", H-5"), 3.27 (1H, d, J = 9.0 Hz, OCH_2), 3.33 (3H, s, OMe), 3.89 (1H, d, J = 9.0 Hz, OCH_2), 4.69 (1H, q, J = 6.0 Hz, H-2), 4.94 (1H, m, H-1"); EIMS *m/z* (rel.

int.): 324 $[M]^+$ (1), 292 (5), 237 (8), 223 (40), 208 (42), 191 (83), 190 (92), 175 (70), 161 (50), 148 (84), 133 (64), 119 (73), 105 (80), 85 (100), 54 (26).

(±)-4-(1'-*Hydroxy*-2'-*methoxymethyl*-2',6'-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol* (**54**)

To a stirred solution of **53** (43.1 g) in EtOH (500 ml) was added pyridinium *p*-toluenesulfonate (4 g), and the mixture was stirred at 55°C for 6.5 hr. The solution was concentrated and the residue was diluted with ether (1 liter), successively washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated. Chromatography of the residual oil on silica gel with hexane-EtOAc (5:1-13:7) afforded 27.4 g (86% yield) of **54** as a mixture of diastereomers. A portion of the mixture was chromatographed on silica gel with hexane-EtOAc (9:1-7:3) to obtain one major diastereomer. ^1H NMR (90 MHz, $CDCl_3$): δ 1.00 (3H, d, J = 6.0 Hz, Me-6), 1.13 (3H, s, Me-2), 1.02-2.06 (6H, m, H-3', H-4', H-5'), 1.47 (3H, d, J = 6.4 Hz, H-1), 2.31 (1H, m, H-6'), 3.09 (1H, d, J = 8.6 Hz, H-8'), 3.37 (3H, s, OMe), 4.05 (1H, d, J = 8.6 Hz, H-8'), 4.47 (1H, q, J = 6.4 Hz, H-2); EIMS *m/z* (rel. int.): 240 $[M]^+$ (1), 222 (4), 207 (6), 190 (20), 175 (55), 163 (25), 148 (48), 133 (32), 126 (41), 105 (47), 93 (58), 82 (100), 69 (52), 54 (15).

(±)-3-(1'-*Hydroxy*-6'-*methoxymethyl*-2',6'-*dimethylcyclohexyl*)-1-*methyl*-2-*propynyl acetate* (**55**)

A mixture of **54** (27.4 g) and Ac_2O (87 g) in pyridine (200 ml) was stirred at room temperature for 13 hr. The mixture was poured into ice-cooled H_2O and extracted with ether. The organic layer was successively washed with 0.1 N HCl, saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (7:3) to afford 29.3 g (91% yield) of **55** as a mixture of diastereomers. A portion of the mixture was chromatographed on silica gel with hexane-EtOAc (20:1-4:1) to obtain a mixture of two major diastereomers (50:50, determined by integrating the 2-methyl singlets in the ^1H NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$): δ 1.03 (3H, d, J = 6.1 Hz, Me-6'), 1.05 (3H, d, J = 6.4 Hz, Me-6'), 1.27-1.93 (12H, m, H-3', H-4', H-5'), 1.22 (3H, s, Me-2'), 1.23 (3H, s, Me-2'), 1.48 (3H, d, J = 6.7 Hz, Me-1), 1.50 (3H, d, J = 6.7 Hz, Me-1), 2.06 (12H, s, OAc), 2.07 (3H, s, OAc), 3.27 (1H, d, J = 9.2 Hz, OCH_2), 3.28 (1H, dd, J = 9.8, 0.3 Hz, OCH_2), 3.35 (3H, s, OMe), 3.35 (3H, s, OMe), 3.54 (1H, d, J = 9.8 Hz, OCH_2), 3.92 (1H, d, J = 9.2 Hz, OCH_2), 5.45 (1H, q, J = 6.7 Hz), 5.49 (1H, q, J = 6.7 Hz); EIMS *m/z* (rel. int.): 222 $[M\text{-}AcOH]^+$ (3), 208 (33), 190 (22), 165 (23), 148 (47), 147 (42), 121 (38), 105 (44), 93 (45), 82 (85), 74 (100), 54 (35).

(±)-3-(2'-*Methoxymethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-1-*methyl*-2-*propynyl acetate* (**56**)

To a stirred solution of **55** (29.3 g) in pyridine (200 ml), a mixture of $POCl_3$ (144 g) and pyridine (180 ml) was added dropwise at 5°C during 1 hr, and the mixture was then heated at 105°C for 6 hr. The mixture was cooled, poured into ice-cooled H_2O, and extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (19:1) to afford 4.3 g (16% yield) of enyne acetate **56**. Further elution with hexane-EtOAc (13:7) allowed the recovery of unreacted **55** (20.9 g). To a stirred solution of **55** (18 g) in pyridine (150 ml), $SOCl_2$ (9.5 g) was added dropwise at -10°C during 20 min. The mixture was stirred at room temperature for 1 hr, and after being quenched with H_2O (200 ml), was extracted with EtOAc. The

organic layer was successively washed with 0.1 N HCl, saturated $NaHCO_3$ and H_2O in that order, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (40:1) to afford 9.8 g (58% yield) of **56**. ^1H NMR (90 MHz, $CDCl_3$): δ 1.01 (3H, s, Me-6'), 1.08-2.21 (6H, m, H-3', H-4', H-5'), 1.51 (3H, d, *J* = 6.2 Hz, H-4), 1.88 (3H, s, Me-2'), 2.05 (3H, s, OAc), 3.16 (1H, d, *J* = 8.4 Hz, OCH_2), 3.32 (3H, s, OMe), 3.40 (1H, d, *J* = 8.4 Hz, OCH_2), 5.59 (1H, q, *J* = 6.2 Hz, H-1); EIMS *m/z* (rel. int.): 204 $[M\text{-}AcOH]^+$ (40), 189 (24), 177 (28), 159 (100), 144 (80), 117 (65), 105 (94), 77 (55), 66 (42), 56 (48).

(±)-(E)-4-(6'-*Methoxymethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*ol* (**57**).

To a stirred solution of **56** (14 g) in THF (200 ml), a mixture of Red-Al (3.4 M in toluene, 160 ml) and THF (40 ml) was added dropwise at 0°C during 40 min under nitrogen. The solution was refluxed for 4.5 hr. A saturated NH_4Cl solution was added to quench the reaction, and the mixture was filtered and extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (4:1) to afford 6.7 g (64% yield) of **57**. ^1H NMR (90 MHz, $CDCl_3$): δ 0.94 (3H, s, Me-6'), 1.06-2.12 (6H, m, H-3', H-4', H-5'), 1.31 (3H, d, *J* = 6.4 Hz, H-1), 1.67 (3H, s, Me-2'), 3.03 (1H, d, *J* = 9.4 Hz, OCH_2), 3.29 (1H, d, *J* = 9.4 Hz, OCH_2), 3.29 (3H, s, OMe), 4.33 (1H, qd, *J* = 6.4, 6.1 Hz, H-2), 5.45 (1H, dd, *J* = 15.4, 6.1 Hz, H-3), 5.99 (1H, br d, *J* = 15.4 Hz, H-4); EIMS *m/z* (rel. int.): 224 $[M]^+$ (1), 206 (8), 179 (100), 161 (31), 147 (4), 135 (11), 121 (73), 119 (29), 105 (27), 93 (38), 91 (23), 79 (18), 71 (14), 54 (4).

(±)-4-(6'-*Methoxymethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*one* (**58**)

A mixture of active MnO_2 (54 g) and **57** (6.6 g) was stirred in CH_2Cl_2 (200 ml) at room temperature for 4 hr. The reaction mixture was filtered, and the resulting cake of MnO_2 was washed with CH_2Cl_2. After being concentrated, the residual oil was chromatographed on silica gel with hexane-EtOAc (19:1) to afford 5.2 g (78% yield) of **58**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.04 (3H, s, Me-6'), 1.33-1.86 (6H, m, H-3', H-4', H-5'), 1.77 (3H, d, *J* = 0.9 Hz, Me-2'), 2.30 (3H, s, H-1), 3.10 (1H, d, *J* = 9.2 Hz, OCH_2), 3.30 (3H, s, OMe), 3.32 (1H, d, *J* = 9.2 Hz, OCH_2), 6.08 (1H, d, *J* = 16.2 Hz, H-3), 7.23 (1H, dd, *J* = 16.2, 0.9 Hz, H-4); EIMS *m/z* (rel. int.): 222 $[M]^+$ (14), 177 (100), 159 (50), 145 (13), 135 (34), 119 (33), 115 (25), 105 (31), 91 (34), 77 (26), 71 (27), 63 (17).

(±)-(2Z,4E *and* 2E,4E)-*methyl* 5-(6'-*methoxymethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*methyl*-2,4-*pentadienoate* (**59**)

A mixture of **58** (5.1 g) and methoxy-carbonyl-methylene-triphenyl-phosphorane (18 g) was stirred at 174°C for 3 hr, before being dissolved in EtOAc (50 ml). To the resulting solution was added hexane (200 ml) to precipitate the triphenylphosphine. The suspension was filtered, and the filtrate was evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (99:1-97:3) to afford 3.3 g (52% yield) of **59** as a mixture of two geometrical isomers (2*Z*:2*E* = 32:68, determined by integrating the 6'-methyl singlets in the ^1H NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$): δ 0.99 (3H, s, Me-6'-2*E*), 1.03 (3H, s, Me-6'-2*Z*), 1.30-2.18 (12H, m, H-3', H-4', H-5'), 1.70 (3H, d, *J* = 0.9 Hz, Me-2'-2*E*), 1.78 (3H, d, *J* = 0.9 Hz, Me-2'-2*Z*), 2.05 (3H, d, *J* = 1.2 Hz, H-6-2*Z*), 2.33 (3H, d, *J* = 1.2 Hz, H-6-2*E*), 3.06 (1H, d, *J* = 8.9 Hz, OCH_2-2*E*), 3.10 (1H, d, *J* = 8.9 Hz, OCH_2-2*Z*), 3.28 (1H, d, *J* = 8.9 Hz, OCH_2-2*E*), 3.30 (3H, s, OMe-2*E*), 3.31 (3H, s, OMe-2*Z*), 3.35 (1H, d, *J* = 8.9 Hz, OCH_2-2*Z*), 3.71 (3H, s, CO_2Me-2*Z*), 3.71 (3H, s, CO_2Me-2*E*), 5.69 (1H, br s, H-2-2*Z*), 5.74 (1H, br s, H-2-2*E*), 6.08 (1H, d, *J* = 16.2 Hz, H-4-2*E*), 6.51 (1H, br d, *J* = 16.2 Hz, H-5-2*E*), 6.55 (1H, br d, *J* = 16.2 Hz, H-5-2*Z*), 7.58 (1H, d, *J* = 16.2 Hz, H-4-2*Z*); EIMS *m/z* (rel. int.): 278 $[M]^+$ (6), 246 (12), 233 (90), 201 (27), 173 (100), 159 (45), 119 (59), 105 (31), 91 (33).

(±)-(2Z,4E *and* 2E,4E)-*methyl* 5-(6'-*methoxymethyl*-2',6'-*dimethyl*-3',4'-*dehydro*-1'-*cyclohexene*-1'-*yl*)-3-*methyl*-2,4-*pentadienoate* (**60**)

To a solution of **59** (3.2 g) in CCl_4 (20 ml) were added NBS (2.9 g) and BPO (34 mg). The mixture was refluxed for 2.3 hr under nitrogen. After cooling the mixture to room temperature, it was filtered, and quinoline (9 ml) was added to the filtrate. The mixture was concentrated, and the residue was heated at 100°C for 1.5 hr under nitrogen. After being cooled to room temperature, the reaction mixture was poured into 1% H_2SO_4 (500 ml) and extracted with ether. The organic layer was successively washed with a saturated $NaHCO_3$ solution and H_2O, dried over Na_2SO_4, and evaporated. The residual oil was chromatographed on silica gel with hexane-EtOAc (39:1) to afford 1.56 g (49% yield) of **60** as a mixture of two geometrical isomers (2*Z*:2*E* = 33:67, determined by integrating the 6'-methyl singlets in the ^1H NMR spectrum). ^1H NMR (400 MHz, $CDCl_3$): δ 1.02 (3H, s, Me-6'-2*E*), 1.06 (3H, s, Me-6'-2*Z*), 1.86 (3H, s, Me-2'-2*E*), 1.94 (3H, s, Me-2'-2*Z*), 1.99 (3H, d, *J* = 1.2 Hz, H-6-2*Z*), 2.06 (4H, m, H-5'), 2.35 (3H, d, *J* = 1.2 Hz, H-6-2*E*), 3.11 (1H, d, *J* = 9.1 Hz, OCH_2-2*E*), 3.13 (1H, d, *J* = 8.6 Hz, OCH_2-2*Z*), 3.30 (1H, d, *J* = 9.1 Hz, OCH_2-2*E*), 3.30 (6H, s, OMe), 3.39 (1H, d, *J* = 8.6 Hz, OCH_2-2*Z*), 3.70 (3H, s, CO_2Me-2*Z*), 3.72 (3H, s, CO_2Me-2*E*), 5.66 (1H, br s, H-2-2*Z*), 5.77 (1H, br s, H-2-2*E*), 5.82 (4H, m, H-3', H-4'), 6.22 (1H, d, *J* = 15.9 Hz, H-4-2*E*), 6.58 (1H, d, *J* = 15.9 Hz, H-5-2*E*), 6.62 (1H, d, *J* = 15.9 Hz, H-5-2*Z*), 7.75 (1H, d, *J* = 15.9 Hz, H-4-2*Z*); EIMS *m/z* (rel. int.): 276 $[M]^+$ (6), 261 (9), 247 (20), 213 (31), 187 (40), 173 (49), 159 (70), 145 (51), 135 (48), 125 (55), 105 (59), 91 (100), 77 (76), 69 (67), 54 (78).

(±)-*8'-Methoxy-ABA* (**4**) *and its* (2E)-*isomer* (**61**), *and* 9'-*methoxy-ABA* (**62**) *and its* (2E)-*isomer* (**63**)

A solution of **60** (1.55 g) and rose bengal (0.23 g) in MeOH (200 ml) was stirred under oxygen while being irradiated with a fluorescent lamp at 24.5°C for 16 hr. The solvent in the reaction mixture was evaporated, the residue was dissolved in MeOH (20 ml), and alumina (active basic, 20 g) was added to the solution. After evaporating the MeOH, hexane (20 ml) was added to the mixture, and the suspension was stirred at room temperature for 2.5 hr before being chromatographed on alumina. Elution with hexane-EtOAc (3:2-1:4) afforded a mixture of starting compound **60** and a crude ester as an oil. The crude ester was purified by chromatography on silica gel with hexane-EtOAc (17:3) to afford 549 mg (32% yield) of a mixture of four isomers. To a solution of this mixture (549 mg) in MeOH was added 1 N NaOH (10 ml). The mixture was stirred at room temperature for 3.5 hr, then diluted with H_2O (100 ml) and washed with hexane. The organic layer was discarded, and the saturated layer was acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was separated in an ODS HPLC column (19 x 150 mm) by eluting with 40% MeOH in 1% AcOH (10

ml/min) to give colorless oils of 45 mg of (±)-**4**, 87 mg of (±)-**61**, 134 mg of (±)-**62**, and 170 mg of (±)-**63**, a portion of each being methylated again with ethereal CH_2N_2 to give the corresponding methyl esters. (±)-**4a**. IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3040-2800, 1704, 1664, 1636, 1604; UV λ_{max} (MeOH) nm (ε): 265 (21,500); EIMS *m/z* (rel. int.): 308 $[M]^+$ (2), 290 (3), 276 (8), 263 (3), 245 (17), 190 (100), 161 (50), 134 (40), 125 (66), 91 (41), 69 (47); HR-EIMS: $[M]^+$ at *m/z* 308.1620 ($C_{17}H_{24}O_5$ requires 308.1624); ^1H NMR, see Table 3. (±)-**61a**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.13 (3H, s, H-9'), 1.93 (3H, d, *J* =1.5 Hz, H-7'), 2.20 (1H, d, *J* = 17.7 Hz, H-5'), 2.27 (1H, d, *J* = 1.2 Hz, H-6), 2.45 (1H, d, *J* = 17.7 Hz, H-5'), 3.23 (1H, d, *J* = 9.5 Hz, H-8'), 3.36 (3H, s, OMe), 3.72 (3H, s, CO_2Me), 3.81 (1H, d, *J* = 9.5 Hz, H-8'), 5.04 (1H, s, OH), 5.85 (1H, br s, H-2), 5.92 (1H, br s, H-3'), 6.01 (1H, d, *J* = 15.6 Hz, H-5), 6.52 (1H, d, *J* = 15.6 Hz, H-4); IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3040-2800, 1708, 1660, 1628, 1612; UV λ_{max} (MeOH) nm (ε): 265 (23,300); EIMS *m/z* (rel. int.): 308 $[M]^+$ (12), 290 (18), 276 (35), 263 (12), 245 (64), 190 (100), 171 (72), 161 (99), 134 (70), 125 (88), 91 (52), 69 (53); HR-EIMS: $[M]^+$ at *m/z* 308.1634 ($C_{17}H_{24}O_5$ requires 308.1624). (±)-**62a**. IR ν_{max} ($CHCl_3$) cm^{-1}: 3460, 3040-2800, 1708, 1664, 1632, 1600; UV λ_{max} (MeOH) nm (ε): 268 (23,000); EIMS *m/z* (rel. int.): 308 $[M]^+$ (2), 290 (2), 276 (6), 263 (3), 245 (11), 190 (85), 161 (36), 134 (34), 125 (100), 91 (26), 69 (26); HR-EIMS: $[M]^+$ at *m/z* 308.1646 ($C_{17}H_{24}O_5$ requires 308.1624); ^1H NMR, see Table 3. (±)-**63a**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.24 (3H, s, H-8'), 1.86 (3H, d, *J* = 1.2 Hz, H-7'), 2.09 (1H, dd, *J* = 17.1, 0.9 Hz, H-5'), 2.29 (1H, d, *J* = 17.1 Hz, H-5'), 2.29 (1H, d, *J* = 1.2 Hz, H-6), 3.09 (1H, d, *J* = 9.2 Hz, H-9'), 3.32 (3H, s, OMe), 3.46 (1H, d, *J* = 9.2 Hz, H-9'), 3.73 (3H, s, CO_2Me), 4.02 (1H, s, OH), 5.87 (1H, br s, H-2), 5.87 (1H, br s, H-3'), 6.18 (1H, d, *J* = 15.3 Hz, H-5), 6.56 (1H, d, *J* = 15.3 Hz, H-4); IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 3040-2800, 1710, 1660, 1625, 1610; UV λ_{max} (MeOH) nm (ε): 268 (22,800); EIMS *m/z* (rel. int.): 308 $[M]^+$ (1), 290 (1), 276 (6), 263 (2), 245 (10), 190 (100), 161 (46), 134 (60), 125 (80), 91 (48), 69 (40); HR-EIMS: $[M]^+$ at *m/z* 308.1609 ($C_{17}H_{24}O_5$ requires 308.1624).

Optical resolution of (±)-**4** *and* -**62**

Racemic mixtures of **4** and **62** were separated into enantiomers in a Chiralpak AD HPLC column with isopropanol-hexane [(±)-**4**, 10:90; (±)-**62**, 6:94] containing 0.1% TFA as the eluent at a flow rate of 1.0 ml min^{-1}. The peaks at t_R 11.6 and 14.4 min of (±)-**4** (16 mg) were collected to give (+)- and (-)-**4** (7.5 and 7.7 mg) with optical purity of 99.9 and 99.5%, respectively, and the peaks at t_R 16.6 and 21.2 min of (±)-**62** (19 mg) were collected to give (+)- and (-)-**62** (8.2 and 9.5 mg) with optical purity of 99.9 and 99.8%, respectively. (+)-**4**: $[\alpha]_D^{26}$ +353.4° (*c* 0.374, $CHCl_3$); CD λ_{ext} (MeOH) nm (Δε): 227 (-28.6), 258 (+26.5), 322 (-1.7); CD λ_{ext} ($CHCl_3$) nm (Δε): 232 (-26.1), 267 (+27.9), 319 (-1.0). (-)-**4**: $[\alpha]_D^{26}$ -355.7° (*c* 0.385, $CHCl_3$); CD λ_{ext} (MeOH) nm (Δε): 229 (+22.0), 259 (-25.5), 320 (+1.9); CD λ_{ext} ($CHCl_3$) nm (Δε): 233 (+22.8), 264 (-25.4), 321 (+3.1). (+)-**62**: $[\alpha]_D^{26}$ +417.6° (*c* 0.408, $CHCl_3$) and +411.4° (*c* 0.408, MeOH); CD λ_{ext} (MeOH) nm (Δε): 227 (-22.9), 263 (+28.0), 321 (-2.6); CD λ_{ext} ($CHCl_3$) nm (Δε): 231 (-35.5), 265 (+42.9), 318 (-2.1). (-)-**62**: $[\alpha]_D^{26}$ -418.7° (*c* 0.473, $CHCl_3$) and -409.5° (*c* 0.473, MeOH); CD λ_{ext} (MeOH) nm (Δε): 225 (+17.1), 264 (-25.5), 319 (+2.4); CD λ_{ext} ($CHCl_3$) nm (Δε): 233 (+25.8), 262 (-35.0), 321 (+4.2).

Bioassays

See Chapter I.2.

I.4 3'-Fluoroabscisic Acid as the Cyclization-Resistant Analogue

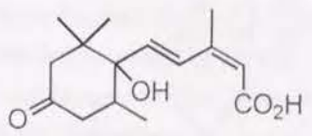
Design Concept

The other way to confer resistance to 8'-HOABA cyclization is to lower the electrophilicity at C-2'. To do this, the energy level of LUMO of the 2'-carbon must be increased.

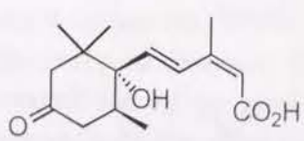
One approach is saturating the 2'-double bond. This analogue, (±)-2',3'-dihydro-ABA (**64**) has been synthesized by Oritani and Yamashita,[96] and its optically pure isomers (**65**, **66**) have been synthesized by Lamb and Abrams,[97,98] but these compounds were not more effective than ABA. Investigation of the metabolism of **65** and **66** has revealed that their 8'-hydroxylated compounds cyclize to the hemiketals by means of nucleophilic addition of the 8'-oxygen to the 4'-carbonyl carbon.[99] That would explain why the dihydro analogues were not strengthened despite blocking the cyclization to PA.

Another approach is increasing the π-electron density at C-2'. This can be performed by reducing the 4'-carbonyl to alcohol and alkane, or by introducing the electron-donating group at C-3'. 1',4'-Diol-ABAs (**67** and **68**, Fig. 15, p. 47)[100] and 4'-deoxy-ABA (**69**)[101] was less effective than ABA, suggesting that the 4'-carbonyl is necessary for activity, indicating that introduction of the electron-donating group to C-3' is preferable to the modification at C-4'.

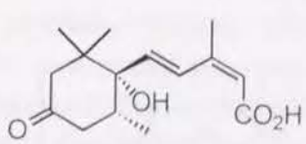
Fluorine is a strong electronegative atom, but the fluorine on sp^2-hybridized carbons pushes π-electrons by repulsing the π-electron and the electrons of the outermost shell of the fluorine atom to increase the π-electron density of the β-carbon. The substitution of fluorine for the 3'-hydrogen of ABA would increase the π-electron density of C-2' by pushing the π-electron at C-3' toward C-2' (Fig. 11), so the author designed 3'-fluoro-ABA (**5**). This modification should confer resistance to the nucleophilic addition of the 8'-oxygen with minimal steric change without removing the functional groups essential for activity.



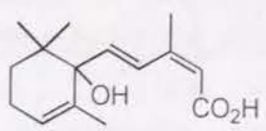
64



65



66



69

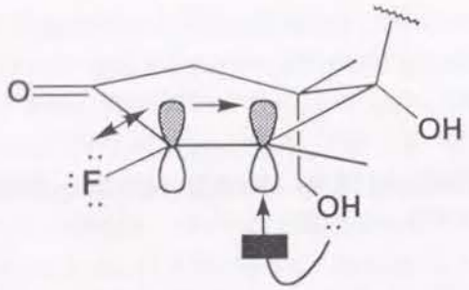


Fig. 11. Electron-donating effect of fluorine.

Results and Discussion

Synthesis

Optically pure (+)-**5** was synthesized from (1'*S*)-(+)-ABA (Fig. 12). The methyl ester **70a** was synthesized stereospecifically by two methods. First, epoxidation of (+)-ABA with alkaline hydrogen peroxide[102] afforded only the oxirane **70**, which was converted to the corresponding methyl ester (**70a**). Second, treating the methyl ester of ABA with hydrogen peroxide and tetrabutylammonium fluoride[103] gave **70a** with the same configuration at C-2' and C-3' as that of the **70a** synthesized first. In the NOESY of **70a**, there was an NOE between the 5- and 7'-protons. This finding suggested that C-7' in **70a** was *cis* to the side chain; **70a** was the α-oxirane. This stereoselective epoxidation may be caused by adding hydrogen peroxide from the less hindered α-face, that is, the opposite side of the side chain (Fig. 12). The reaction of **70a** with a complex of hydrogen fluoride and *N*-ethyldiisopropylamine[104] afforded the fluoro-olefin **5a**, probably via the fluorohydrin **71**. Hydrolysis of **5a** with alkali gave the free acid (+)-**5**.

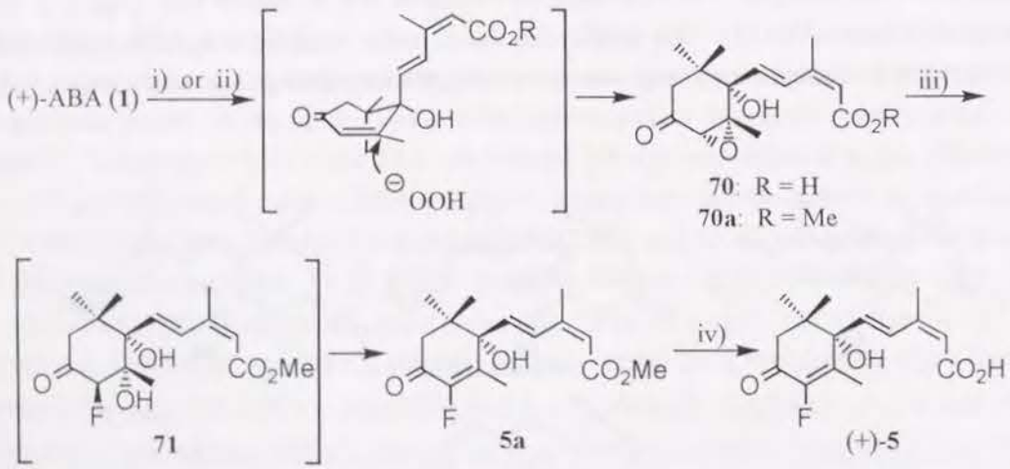


Fig. 12. Synthesis of (+)-**5**.

i) a) H_2O_2, NaOH b) CH_2N_2 ii) a) CH_2N_2 b) H_2O_2, Bu_4NF iii) Et(*i*-Pr)$_2$N(HF)$_3$
iv) NaOH.

The existence of a fluorine at C-3' in (+)-**5** was proven by the disappearance of the 3'-proton in ^{1}H NMR and the appearance of ^{19}F signal and ^{13}C signals split by coupling with fluorine. The optical purity of (+)-**5** was confirmed by HPLC with a chiral column that gave only one peak under the conditions that give two separate peaks for the racemic **5**. The absolute configuration at C-1' in (+)-**5** was determined to be *S*, as it had the same Cotton effects in the CD spectrum as (+)-ABA.[77]

The ^{13}C signal of the 2'-carbon in (+)-**5** appeared in a field higher by 24.7 ppm than that of (±)-ABA.[105] The ^{13}C chemical shifts correlates to electron density in a carbon atom,[106] so this result showed that the electron density of C-2' in (+)-**5** is higher than that in ABA as expected. In contrast to C-2', C-3' in (+)-**5** shifted toward a low field by 23.9 ppm compared to that in ABA by repulsion of the π-electron and the electrons of the outermost shell of fluorine atom.

Biological activity

The inhibitory activities of (+)-**5** in the lettuce seed germination and the elongation of the second leaf sheath of rice seedling were tested (Table 5). The activity of analogue (+)-**5** was slightly higher than that of (+)-ABA in the lettuce seeds, and was almost equal to that of (+)-ABA in the rice seedlings. If the metabolism was suppressed, then the activity should be strengthened. Therefore, this result suggested that (+)-**5** was metabolized in a manner similar to ABA.

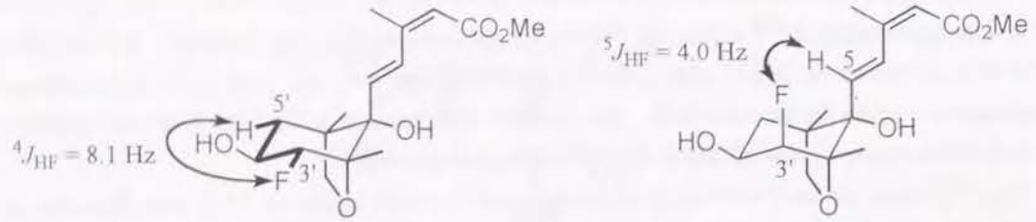
Metabolism in bean shoots

To understand how (+)-**5** is metabolized, it was fed to bean shoots through the cut ends via transpiration stream for three days. The plant extract was partitioned under acidic conditions to give EtOAc and aqueous extracts.

Identification of 3'-fluoro-DPAs in the EtOAc extract. The EtOAc extract was treated with diazomethane to methylate the free metabolites. This methylated extract exhibited three major peaks by gas-liquid chromatography (GLC) using a XE-60 column and an electron-capture detector with high selectivity and sensitivity for ABA and its metabolites.[107] The retention time of the major peak agreed with that of **5a** in GLC analyses using not only XE-60 but also OV-17 and SE-30. Therefore, the compound corresponding to this peak was identified as **5a** derived from unmetabolized (+)-**5**. Compounds giving the other two peaks were purified by column chromatography on silica gel followed by ODS. Finally, two compounds were isolated by HPLC. The more polar compound (**75a**) corresponded to the peak with the longer retention time in GLC with XE-60, whereas the less polar compound (**76a**) corresponded to the peak with the shorter retention time. Compounds **75a** and **76a** showed a molecular ion at *m/z* 314. In

Table 5. The IC_{50} Values for (+)-ABA and (+)-**5** in Bioassays

Compound	IC_{50} in assay	
	Rice seedling elongation (μM)	Lettuce seed germination (μM)
(+)-ABA	2.1	5.0
(+)-**5**	1.9	2.0



75a **76a**

Fig. 13. Structures of **75a** and **76a**, and the long-range ^{1}H-^{19}F couplings in ^{1}H NMR.

the ^{1}H NMR analysis, they showed similar signals to the methyl ester of DPA, except for those of the 3'-protons, which appeared at a field lower by about 2 ppm than those of the methyl ester of DPA[108] and which were split by large couplings over 50 Hz (Table 6). The presence of the fluorine at C-3' in each compound was confirmed by a ^{19}F signal which appeared as a double doublet split by couplings with the 3'- and 4'-protons (Table 7). These findings suggested that these compounds were the methyl ester of 3'-fluoro-DPAs which were epimeric at C-3'. The absolute configuration at C-3' was determined on the basis of the long-range ^{1}H-^{19}F couplings in ^{1}H NMR. Compound **75a** possessed the 5'*pro-R*-proton with large long-range ^{1}H-^{19}F coupling (8.1 Hz), which depends on the W-arrangement that can occur only when the 3'-fluorine is in the equatorial α-position (Fig. 13). These findings showed that **75a** was the methyl ester of 3'α-fluoro-DPA (**75**). Compound **76a** had a 5-proton exhibiting the ^{1}H-^{19}F coupling (4.0 Hz) although it was separated by five σ-bonds, indicating that the 5-proton is spatially close to the 3'-fluorine. This type of relationship between the 5-proton and the 3'-fluorine is formed when the 3'-fluorine is in the axial β-position (Fig. 13), so **76a** was the methyl ester of 3'β-fluoro-DPA (**76**). Considering that the configuration at C-1' in the precursor (+)-**5** is the same as that of natural (+)-ABA, the absolute configuration of all the asymmetric carbons in **75a** was elucidated as (1'*S*,2'*S*,3'*R*,4'*S*,6'*R*), while that of **76a** as (1'*S*,2'*S*,3'*S*,4'*S*,6'*R*).

Identification and properties of 3'-fluoro-8'-HOABA and 3'-fluoro-PAs in the EtOAc extract. The presence of **75** and **76** suggested that 3'α- and 3'β-fluoro-PAs (**73** and **74**) which are the immediate precursors of **75** and **76**, respectively, must be in the EtOAc extract. These compounds seemed to be minor peaks in GLC with XE-60. In order to investigate the presence of the methyl esters **73a** and **74a** in the methylated EtOAc extract, **73a** and **74a** as the standard samples were prepared from **75a** and **76a**, respectively, by Jones oxidation.[109] These structures were ascertained by MS and ^{1}H and ^{19}F NMR data (Tables 6, 7). Immediately after purification, these compounds gave single peaks in HPLC and single spots in TLC. When **73a** was left for several hours at 25°C, however, half of it was converted into two compounds. The minor compound was identified as **74a** from the retention time in HPLC and its R_f in TLC. The major one **72a** exhibited a peak with a retention time intermediate between **73a** and **74a** in HPLC and a spot with the lowest t_R among three compounds in TLC. Isolated **72a** gave no signal corresponding to the 3'-proton of **73a**, and showed a signal for the 7'-protons at a field lower by 0.51 ppm than that of **73a** in ^{1}H NMR (Table 7). In ^{19}F NMR, a singlet signal was observed at -134.8 ppm (Table

Table 6. ^{1}H HMR Data for **72a**-**76a** in $CDCl_3$

H	**72a***	**73a****	**74a****	**75a****	**76a****
2	5.78 (br s)	5.85 (br s)	5.78 (br s)	5.79 (br s)	5.75 (br s)
3	7.90 (d, 16.0)	8.24 (d, 16.0)	8.09 (d, 15.8)	8.10 (d, 16.0)	8.00 (d, 16.0)
5	6.00 (d, 16.0)	6.20 (d, 16.0)	6.30 (dd, 15.8, $^5J_{HF}$=3.2)	6.13 (d, 16.0)	6.52 (dd, 16.0, $^5J_{HF}$=4.0)
6	2.02 (d, 1.3)	2.04 (d, 1.2)	1.99 (d, 1.3)	2.03 (d, 1.1)	2.04 (d, 1.2)
3'	—	4.77 (d, $^2J_{HF}$=46.8)	4.31 (dd, 1.1, $^2J_{HF}$=48.0)	4.33 (dd, 7.7, $^2J_{HF}$=50.0)	4.55 (ddd, 4.6, 1.2, $^2J_{HF}$=51.5)
4'	—	—	—	4.23 (m)	4.22 (m)
5'-*proR*	2.45 (dd, 17.5, $^4J_{HF}$=1.5)	2.52 (dd, 17.4, $^4J_{HF}$=5.2)	2.53 (dd, 19.0, 1.1)	1.98 (ddd, 13.8,4.7, $^4J_{HF}$=8.1)	1.95 (ddd, 14.1,4.0, 1.2)
5'-*proS*	2.54 (dd, 17.5, $^4J_{HF}$=3.9)	2.60 (dd, 17.4, 2.6)	2.66 (ddd, 19.0,2.3, $^4J_{HF}$=0.6)	1.62 (ddd, 13.8, 11.0, 2.4)	1.70 (ddd, 14.1, 11.0, 1.9)
7'	1.91 (d, $^4J_{HF}$=3.6)	1.40 (d, $^4J_{HF}$=1.8)	1.37 (d, $^4J_{HF}$=2.6)	1.29 (d, $^4J_{HF}$=2.0)	1.34 (d, $^4J_{HF}$=1.0)
8'	3.62 (dd, 11.2, 5.0)	3.72 (d, 8,2, H_S)	3.71 (d, 7.7, H_S)	3.75 (d, 8.0, H_S)	3.68 (d, 8.7, H_S)
	3.99 (dd, 11.2, 5.0)	3.98 (dd, 8.2, 2.6, H_R)	4.06 (ddd, 7.7, 2.3, $^5J_{HF}$=1.9, H_R)	3.88 (dd, 8.0, 2.4, H_R)	3.86 (ddd, 8.7, 1.9, $^5J_{HF}$=4.0, H_R)
9'	1.13 (s)	1.08 (s)	1.04 (s)	0.99 (s)	0.96 (s)
1'-OH	4.59 (s)	2.20 (s)	2.15 (s)	2.01 (s)	1.93 (s)
4'-OH	—	—	—	2.25 (d, 3.5)	1.83 (dd, 3.2, $^4J_{HF}$=10.1)
8'-OH	2.34 (dd, 5.0, 5.0)	—	—	—	—
CO_2Me	3.71 (s)	3.74 (s)	3.73 (s)	3.73 (s)	3.72 (s)

* 300 MHz. ** 500 MHz. Values for the chemical shifts are δ (ppm). Multiplicity of signals and coupling constants (Hz) are shown in parentheses.

Table 7. ^{19}F NMR (282 MHz) Data of (+)-**5** in Acetone-d_6 and **72a**-**76a** in $CDCl_3$

Compound	^{19}F chemical shift	Multiplicity	Coupling constants
(+)-**5**	-135.4	s	
72a	-134.8	s	
73a	-204.5	d	$^2J_{FH}$ = 46.8 Hz
74a	-175.0	d	$^2J_{FH}$ = 48.0 Hz
75a	-133.1	dd	$^2J_{FH}$ = 50.0 Hz, $^3J_{FH}$ = 22.4 Hz
76a	-197.8	dd	$^2J_{FH}$ = 51.5 Hz, $^3J_{FH}$ = 25.8 Hz

Values for the chemical shifts are shown by ppm from CCl_3F as the internal standard.

6). MS revealed that the molecular weight was 312, which agreed with those of **73a** and **74a**. These data showed that **72a** was the methyl ester of 3'-fluoro-8'-HOABA (**72**). Some of compound **72a** was spontaneously and easily converted to **73a** and **74a**, while **74a** was relatively stable and converted very slowly to **72a** and **73a**. Each compound was converted finally into an equilibrium mixture of **72a**, **73a** and **74a** at 25°C in the ratio of 7:6:1, as determined by HPLC (Fig. 14). This would be caused by the weakened electrophilicity of C-2' by the effect of fluorine as expected. Such spontaneous back-isomerization of the cyclized metabolite cannot be observed between 8'-HOABA and (-)-PA.

The stability of **74a** compared with **72a** and **73a** suggests that the energy barrier against the conversion of **74a** to other isomers is higher than that of **72a** and **73a**. The small proportion of **74a** in equilibrium and the high energy barrier against the conversion indicated that the isomerization to **74a** is unfavorable both thermodynamically and kinetically. The thermodynamic instability of **74a** would be caused by 1,3-diaxial steric repulsion between the fluorine and the side chain. The **72a**/**74a** and **73a**/**74a** ratios in equilibrium suggest that **74a** is less stable by 1 kcal mol^{-1} than **72a** and **73a**. The transition state for enolization-ketonization between **74a** and the enol **77a** would be less stable than that between **73a** and **77a**. This is because there is less σ-π interaction between the σ-electron at the 3'-proton and the π-electron at the 4'-carbonyl when the entering or leaving proton has an equatorial orientation as opposed to an alternative axial orientation.[110] This would cause a high energy barrier against the isomerization of **74a**.

Compounds **72a**, **73a** and **74a** afforded extremely broad peaks in GLC with XE-60, probably because of the fast interconversion at high temperature and the inappropriate liquid phase or support. Detection of these compounds in the methylated EtOAc extract was thus difficult in GLC with XE-60. Apiezon Grease L® gave better separation than silicon. GLC of **72a**, **73a** and **74a** using Apiezon Grease L® gave the same chromatograms, which consisted of four peaks in the ratio of about 6:1:1:6, although they were still broad and overlapping. All four peaks gave the ion at *m/z* 312 in GC-MS analysis, suggesting that they were not degradation products but isomers converted by the high temperature. The two high peaks among the four in GLC will correspond to **72a** and **73a** although it is unknown which is which. One of the two small peaks will correspond to **74a**, and the other small one may correspond to the enol **77a**. The lack of the enol **77a** in HPLC using a mixture of MeOH and water as the solvent can be explained by its instability in polar solvents. Enols forming intramolecular hydrogen bonds are stabilized in apolar solvents especially in the gas phase, but they are labile in polar solvents such as MeOH and chloroform.[111] The enol **77a**, which can form intramolecular hydrogen bonding between the 4'-hydroxyl and 3'-fluoro groups, may have these features. Metabolites **72**-**74** would also be interconverted via **77** *in vivo* (Fig. 14).

GLC of the methylated EtOAc extract with Apiezon Grease L® confirmed the equilibrium mixture of **72a**, **73a** and **74a**. HPLC of the fractions purified from the extract revealed peaks with the same retention time as the standard samples of **72a** and **73a**. After isolation, these compounds were identified with **72a** and **73a** from the spectral data. A peak corresponding to **72a** was not found due to the small amount and masking by overlapping peaks, but it must also be in the extract. The ratio among unmetabolized (+)-**5**, metabolites **72**-**74**, metabolite **75**, and metabolite **76** in the EtOAc extract was

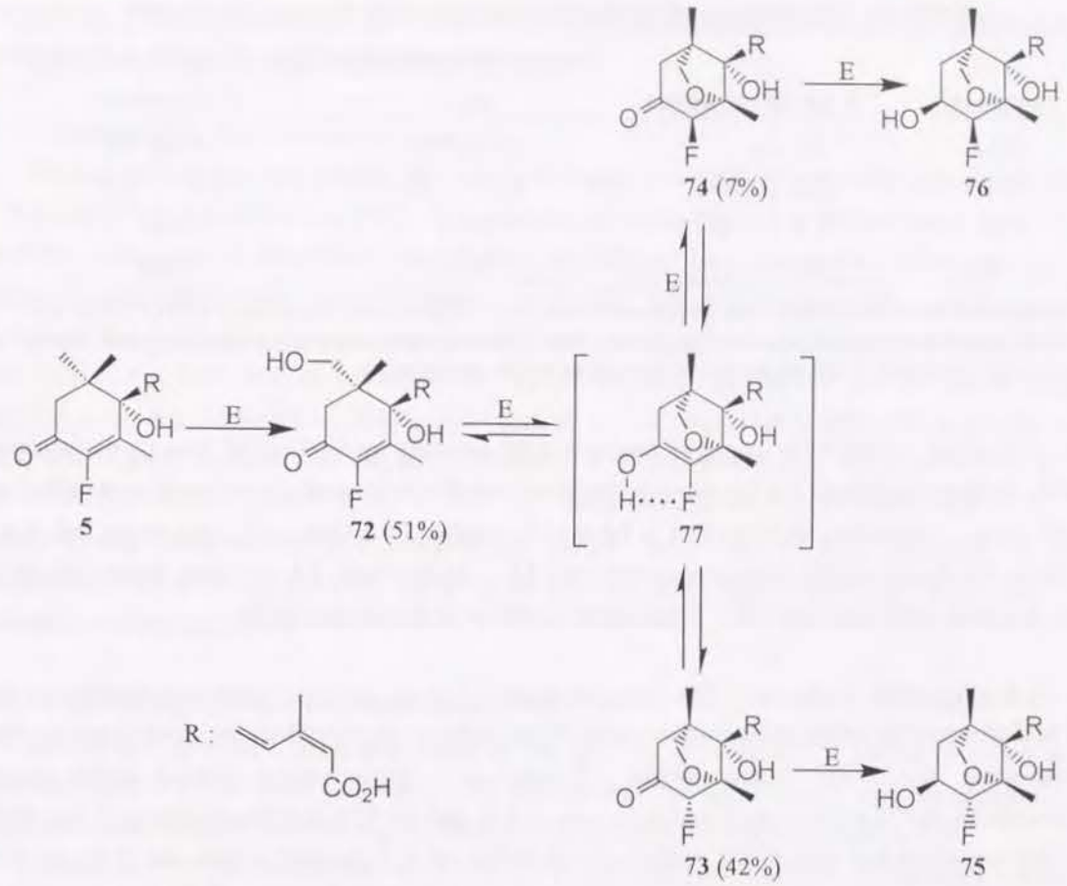


Fig. 14. Supposed oxidation pathway in the metabolism of (+)-**5** in bean shoots.
"E" means the step catalyzed by an enzyme. Percentages in the parentheses are the compositions of methyl esters **72a**, **73a** and **74a** in the final equilibrium in methanol.

estimated to be 1:2:1:1 from the contents of their methyl esters determined by GLC analyses using XE-60 and Apiezon Grease L® (Table 8).

The slight increase of biological activity of (+)-**5** in the lettuce seeds may be caused by the stability of **78** which would maintain the activity, or else by the higher affinity of (+)-**5** for the receptor involved in the inhibitory activity in lettuce seed germination than ABA. However, the stability of **72** is not enough to give (+)-**5** prominently high activity since **72** could be metabolized to **73** and **74**, and then to **75** and **76**.

Involvement of enzymes in the cyclizing step. The ratio between metabolites **75** and **76**, 1:1, was inconsistent with the **73a**/**74a** ratio (6:1) in equilibrium. If the cyclization of **74** occurs nonenzymatically, the ratio of **75** to **76** must be 6:1. This discrepancy supports the participation of the cyclization enzyme in this conversion[57]; metabolite **72** would be enzymatically converted to **74**, which then may be isomerized to **72** and **73** owing to equilibrium before being reduced to **76**, or some of **72** may be isomerized depending upon equilibrium to **73** before being enzymatically cyclized (Fig. 14). The tendency of the 4'-

Table 8. The Recovery of (+)-**5** and its Metabolites **72-76** from Bean Shoots

Compound	In the EtOAc extract	Released by hydrolysis of the conjugate fraction with alkali	Released by hydrolysis of the conjugate fraction with β-glucosidase
(+)-**5**	0.6 mg	0.3 mg (3)	0.3 mg (3)
72-74	1.2 (13)	0.2 (2)	0.2 (2)
75	0.6 (7)	trace	trace
76	0.6 (7)	trace	trace

Compounds were analyzed as the methyl ester and their weights were determined in GLC with 1% XE-60 and 1% Apiezon Grease L® columns. The ratios of conversion of the applied (+)-**5** (9 mg) into each metabolite were presented in percentage in the parentheses.

carbonyl to accept nucleophiles would be enhanced more intensely by the 3'-axial, than by the equatorial fluorine, because the former can have a σ-π interaction with the π bond of the carbonyl more effectively than the latter. Therefore, the 4'-carbon in **74** may be attacked by hydride ions more easily than that in **73**, that is, **74** may be metabolized more quickly than **73**. Alternatively, **74** may have higher affinity for the active site of a reductase than **73**. These might contribute to the increase of **76**.

Hydrolysis of the conjugates. The conjugate fraction from the aqueous extract was hydrolyzed with alkali or β-glucosidase before being extracted with EtOAc under acidic conditions and methylated for GLC analysis using XE-60 and Apiezon Grease L® columns. These extracts yielded similar results. Compounds **5a** and **72a-74a** were found in the ratio of 3:2, and the **75a** and **76a** levels were low (Table 8). This suggested that most of the conjugated metabolites are C-1-glucosyl esters, not C-1'- or C-8'-glucosides. The total amount of the conjugated metabolites was less than that of the free metabolites, indicating that the oxidation pathway mostly concerns (+)-**5** in bean shoots.

In conclusion, the introduction of fluorine at C-3' in ABA increased the electron density at C-2' so much that the methyl esters of 3'-fluoro-8'-HOABA and 3'-fluoro-PAs coexisted at equilibrium by the partial resistance of C-3' to attack by the 8'-hydroxyl group because of the effect the fluorine atom at C-3' has on C-2'. This finding shows that introducing an electron donating group at C-3' is effective to reduce the nucleophilic addition of the 8'-hydroxyl group.

Experimental

General procedures

The ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal standard at 300 or 500 MHz for ^{1}H and 125 MHz for ^{13}C using Bruker ARX500 and AC300 instruments. For clarity, the atoms of all the compounds with the carbon skeleton of ABA were numbered as in ABA in the assignment of peaks. ^{19}F NMR spectra were recorded at 282 MHz on a Bruker AC300. ^{19}F chemical shifts were reported in ppm from CCl_3F as an internal reference and the higher field resonance from the CCl_3F signal was assigned as negative. 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.

2'α,3'α-Dihydro-2'α,3'α-epoxyabscisic acid (**70**)

To a stirred solution of (+)-ABA (200 mg, 0.76 mmol) in MeOH (20 ml) were added 30% H_2O_2 (0.2 ml) and 6 N NaOH (0.6 ml) at 0°C. The mixture was stirred for 8 hr at 0°C and made up to 40 ml with H_2O. After lowering the pH to 2 with 3 N HCl, the mixture was extracted with EtOAc (30 ml x 3). The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (12 g) with hexane-EtOAc-AcOH (80:20:3) to afford **70** (114 mg, 54% yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.94 (3H, s, H_3-9'), 0.99 (3H, s, H_3-8'), 1.39 (3H, s, H_3-7'), 1.83 (1H, d, J = 14.7 Hz, H-5'), 2.07 (3H, s, H_3-6), 2.67 (1H, s, OH), 2.84 (1H, d, J = 14.7 Hz, H-5'), 3.30 (1H, s, H-3'), 5.79 (1H, s, H-2), 6.11 (1H, d, J = 15.8 Hz, H-5), 8.03 (1H, d, J = 15.8 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 253 (15,700); $[\alpha]_D^{26}$ +6° (MeOH, *c* 0.41); EIMS *m/z* (rel. int.): 280 $[M]^+$ (6), 262 $[M - H_2O]^+$ (4), 251 (22), 206 (11), 196 (25), 178 (33), 151 (50), 135 (20), 121 (42), 111 (76), 94 (26), 85 (100); HR-EIMS: $[M]^+$ at *m/z* 280.1307 ($C_{15}H_{20}O_5$ requires 280.1311).

Methyl 2'α,3'α-dihydro-2'α,3'α-epoxyabscisate (**70a**)

Method i). Ethereal CH_2N_2 was added to **70** (100 mg, 0.36 mmol) in MeOH (3 ml) at room temperature until a yellow color persisted. The mixture was left for 1 hr at room temperature, and concentrated under reduced pressure to give **70a** (105 mg, quantitative yield) as a colorless oil.

Method ii).[103] The methyl ester of (+)-ABA was prepared from (+)-ABA by the same method as method i). To a stirred mixture of the methyl ester of (+)-ABA (100 mg, 0.36 mmol) in dimethyl sulfoxide and 30% H_2O_2 (180 ml) was added dropwise, tetrabutylammonium fluoride (1.0 M tetrahydrofuran solution, 2.36 ml, 2.36 mmol) at room temperature under nitrogen. The mixture was stirred at the same temperature for 5 hr. H_2O (30 ml) was added and the product was extracted with EtOAc (20 ml x 3). The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (2 g) with hexane-EtOAc (8:2) to give **70a** (44 mg, 41% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (3H, s, H_3-9'), 0.99 (3H, s, H_3-8'), 1.39 (3H, s, H_3-7'), 1.83 (1H, dd, J = 14.6 and 0.7 Hz, H-5'), 2.03 (3H, d, J = 1.1 Hz, H_3-6), 2.83 (1H, d, J = 14.6 Hz, H-5'), 2.87 (1H, d, J = 0.7 Hz, OH), 3.29 (1H, br s, H-3'), 3.34 (3H, s, OMe), 5.77 (1H, br s, H-2), 6.06 (1H, d, J = 15.8 Hz, H-5), 8.07 (1H, d, J = 15.8 Hz, H-4); EIMS *m/z* (rel. int.): 294 $[M]^+$ (5), 276 $[M - H_2O]^+$ (2), 265 (15), 238 (12), 210 (17), 206 (17), 195 (27), 178 (41), 165 (17), 151 (56), 135 (23), 125 (100); HR-EIMS: $[M]^+$ at *m/z* 294.1464 ($C_{16}H_{22}O_5$ requires 294.1467).

Methyl 3'-fluoroabscisate (**5a**)

A stirred solution of **70a** (21 mg, 0.071 mmol) in *N*-ethyldiisopropylamine (0.5 ml) and *N*-ethyldiisopropylamine tris(hydrofluoride)[104] (40 ml) was heated at 145°C for 4 hr. The solution was poured into 3N HCl (20 ml) and extracted with EtOAc (20 ml x 3). The organic layer was washed with

saturated aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (2 g) to give **5a** (6 mg, 28% yield) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 1.04 (3H, s, H3-9'), 1.14 (3H, s, H3-8'), 1.87 (3H, d, $^4J_{HF}$ = 3.5 Hz, H3-7'), 2.02 (3H, d, J = 1.1 Hz, H3-6), 2.11 (1H, s, OH), 2.43 (1H, dd, J = 16.9 and $^4J_{HF}$ = 4.4 Hz, H-5'*pro-R*), 2.49 (1H, d, J = 16.9 Hz, H-5'*pro-S*), 3.71 (3H, s, OMe), 5.78 (1H, br s, H-2), 6.09 (1H, d, J = 16.1 Hz, H-5), 7.88 (1H, d, J = 16.1 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 262 (23,100); IR ν_{max} ($CHCl_3$) cm^{-1}: 3500, 2950, 1690, 1650, 1630, 1600; EIMS *m/z* (rel. int.): 296 $[M]^+$ (4), 278 $[M - H_2O]^+$ (5), 264 (6), 246 (4), 223 (6), 208 (70), 183 (46), 180 (50), 165 (15), 152 (55), 125 (100); HR-EIMS: $[M]^+$ at *m/z* 296.1440 ($C_{16}H_{21}O_4F$ requires 296.1424).

(+)-3'-*Fluoroabscisic acid* (**5**)

To a solution of **5a** (42 mg, 0.14 mmol) in MeOH (1 ml) was added 1N NaOH (0.8 ml). The mixture was stirred at room temperature for 3 hr, then H_2O (30 ml) was added. After lowering the pH to 2 with 3N HCl, the mixture was extracted with EtOAc (20 ml x 3). The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on silica gel (16 g) under pressure to afford (±)-**5** (38 mg, 95% yield) as a white amorphous solid. ^1H NMR (500 MHz, acetone-d_6): δ 1.07 (3H, s, H3-9'), 1.11 (3H, s, H3-8'), 1.85 (3H, d, $^4J_{HF}$ = 3.5 Hz, H3-7'), 2.07 (3H, d, J = 1.0 Hz, H3-6), 2.33 (1H, br d, J = 16.8, H-5'), 2.63 (1H, d, J = 16.8 Hz, H-5'), 5.78 (1H, br s, H-2), 6.39 (1H, d, J = 16.0 Hz, H-5), 7.93 (1H, d, J = 16.0 Hz, H-4); ^{13}C NMR (125 MHz, acetone-d_6): δ 10.4 (d, $^3J_{CF}$ = 4.7 Hz, C-7'), 21.0 (C-6), 23.2 (C-8'), 24.3 (C-9'), 42.1 (C-6'), 49.3 (d, $^3J_{CF}$ = 4.0 Hz, C-5'), 79.5 (d, $^3J_{CF}$ = 5.1 Hz, C-1'), 118.7 (C-2), 128.8 (C-4), 137.4 (d, $^4J_{CF}$ = 2.5 Hz, C-5), 138.3 (d, $^2J_{CF}$ = 6.4 Hz, C-2'), 150.7 (C-3), 151.0 (d, $^1J_{CF}$ = 258.4 Hz, C-3'), 167.0 (C-1), 189.0 (d, $^2J_{CF}$ = 20.5 Hz, C-4'), : $[\alpha]_D^{17}$ +264° (MeOH, *c* 0.32); CD λ_{ext} (MeOH) nm (Δε): 327 (-1.5), 260 (+27.2), 231 (-21.6); UV λ_{max} (MeOH) nm (ε): 247 (24,300); EIMS *m/z* (rel. int.): 282 $[M]^+$ (6), 264 $[M - H_2O]^+$ (22), 249 (15), 239 (15), 223 (13), 208 (100), 183 (85), 180 (68), 165 (22), 152 (66), 124 (17), 111 (48); HR-EIMS: $[M]^+$ at *m/z* 282.1263 ($C_{15}H_{19}O_4F$ requires 282.1267). The ^1H, ^{13}C and ^{19}F NMR data are listed in Tables 6 and 7.

(±)-3'-*Fluoroabscisic acid* [(±)-**5**] *and HPLC analysis with a chiral column*

In the same manner as (+)-**5**, (±)-**5** was synthesized from (±)-ABA. The racemate (±)-**5** gave two separate peaks with retention times of 8.2 and 9.4 min in HPLC with a Chiralcel OD column (4.6 x 250 mm, Daicel; solvent, 11% *i*-PrOH in hexane containing 0.1% TFA; flow rate, 1.0 ml min^{-1}; detection, 254 nm). Optically active (+)-**5** afforded only one peak at t_R 8.2 min under the same conditions.

Plant material and application of (+)-**5**[112]

Bean plants (*Phaseolus vulgaris* L. cv. Kentucky Wonder) were grown in a greenhouse for 20 days until the primary leaves were mature and the first trifoliate leaf half expanded. The temperature in the greenhouse was maintained below 23°C during the day, and above 15°C during the night. To introduce compounds via the transpiration stream, the stems were cut at 0.5 cm above root apex under degassed water. For application to bean shoots, an acetone (0.6 ml) solution of (+)-**5** (9 mg) was mixed with about

50 ml of Tween 20 and filled up to 150 ml with H_2O. Thirty shoots were placed in the solution for three days under continuous light (5000 lux) at 25°C. The vessel was covered with a sheet of black polyethylene to prevent photo-isomerization. HPLC analysis of the solutions after the application showed that 8.5 mg of (+)-**5** was incorporated into the shoots.

Extraction from bean shoots

Plant samples (66 g, fresh weight) that incorporated (+)-**5** were frozen in liquid nitrogen, pulverized and extracted with MeOH (400 ml) containing 10 mg ml^{-1} 2,6-di-*tert*-butyl-4-methylphenol for three days. After filtration, the tissue residue was washed several times with methanol. The combined filtrates were concentrated to a small volume and were brought to 250 ml with H_2O. After lowering the pH to 2 with 3 N HCl, the solution was extracted with EtOAc. The organic layer was washed with H_2O and concentrated under reduced pressure to give the EtOAc extract (334 mg). The aqueous layer, after adjusting the pH to 5, was concentrated under reduced pressure to give the aqueous extract (38 mg).

Isolation of metabolites from the EtOAc extract

To a solution of the EtOAc extract (310 mg) in EtOAc (2 ml) was added ethereal CH_2N_2 until a yellow color persisted. The mixture was left for 0.5 hr at room temperature, then the solvent was removed. The residue was applied to a column packed with silica gel (15 g) and eluted with 10, 20, 30, 40, 60 and 80% in toluene. The 40 and 60% EtOAc fractions were combined and concentrated under reduced pressure. The residue was purified further by chromatography using ODS (AM120-S50, YMC, 16g) with 60% MeOH in H_2O to give 2 mg of a crude oil. The crude oil (2 mg) was injected into an HPLC column (mBondasphere 5m C18-100Å, 19 x 150 mm, Waters; solvent, 55% MeOH; flow rate, 10 ml min^{-1}; detection, 254 nm). The materials with t_R 8.2 and 13.9 min were collected to give **75a** (0.3 mg) and **76a** (0.3 mg) as colorless oils, respectively. The eluate containing other materials was injected into the HPLC column again. The material with t_R 9.8 min was collected to give **72a** (0.1 mg) as a colorless oil. The 30% EtOAc fraction was concentrated, suspended in MeOH-H_2O (9:1), then chromatographed in portions over Sep-Pak® C18 (original type, Millipore) using 30, 60 and 80% MeOH. The 60% MeOH fraction was injected into a HPLC column (AQ 311, 6 x 100 mm, YMC; solvent, 55% MeOH; flow rate, 2 ml min^{-1}; detection, 254 nm). The material with t_R 2.7 min was collected to give **73a** (0.1 mg) as a colorless oil. **75a**: $[\alpha]_D^{21}$ -80° (MeOH, *c* 0.014); UV λ_{max} (MeOH) nm (ε): 264 (22,000); EIMS *m/z* (rel. int.): 314 $[M]^+$ (12), 296 $[M - H_2O]^+$ (16), 282 (5), 264 (6), 237 (15), 221 (6), 195 (7), 177 (14), 163 (21), 154 (34), 135 (30), 122 (100); HR-EIMS: $[M]^+$ at *m/z* 314.1522 ($C_{16}H_{23}O_5F$ requires 314.1529). **76a**: $[\alpha]_D^{21}$ -197° (MeOH, *c* 0.0158); UV λ_{max} (MeOH) nm (ε): 267 (22,000); EIMS *m/z* (rel. int.): 314 $[M]^+$ (8), 296 $[M - H_2O]^+$ (17), 282 (6), 264 (6), 237 (8), 219 (5), 195 (10), 177 (14), 163 (24), 154 (29), 135 (31), 125 (100); HR-EIMS: $[M]^+$ at *m/z* 314.1502 ($C_{16}H_{23}O_5F$ requires 314.1529). **72a**: UV λ_{max} (MeOH) nm (ε): 263 (24,000); EIMS *m/z* (rel. int.): 312 $[M]^+$ (6), 294 $[M - H_2O]^+$ (13), 280 (14), 264 (24), 249 (10), 239 (15), 221 (10), 208 (30), 199 (18), 179 (23), 163 (14), 152 (29), 125 (100); HR-EIMS: $[M]^+$ at *m/z* 312.1376 ($C_{16}H_{21}O_5F$ requires 312.1373). **73a**: UV λ_{max} (MeOH) nm (ε): 263 (18,500); EIMS *m/z* (rel. int.): 312 $[M]^+$ (34), 294 $[M - H_2O]^+$ (5), 292 (3), 280 (14), 264 (8), 249 (5), 239 (10), 221 (10), 208 (14), 177 (24), 163 (31), 154

(32), 135 (38), 125 (100); HR-EIMS: $[M]^+$ at *m/z* 312.1366 ($C_{16}H_{21}O_5F$ requires 312.1373). The ^1H and ^{19}F NMR spectral data are listed in Tables 6 and 7. The value of the optical rotation of **72a** and **73a** was too small to be measured.

Hydrolysis of the conjugate fraction with alkali and β-glucosidase

The aqueous extract (38 mg) was roughly purified by chromatography on ODS (16 g). The first fraction that eluted with H_2O was discarded, and the second MeOH fraction was concentrated to give the conjugate fraction (34 mg). To the conjugate fraction (15 mg) was added 1 N NaOH (45 ml), and the mixture was left at room temperature for 6 hr. The conjugate fraction (15 mg) was made up in 0.05 M citrate-sodium citrate buffer (pH 5.0, 30 ml), and β-glucosidase (EC 3.2.1.21, Sigma G0395, 235 units) was added. The mixture was incubated for 6 hr at 37°C. The residual conjugate fraction (4 mg) was not hydrolyzed. After lowering the pH to 2 with 3 N HCl, these solutions were extracted with EtOAc, respectively. The organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4 and concentrated to give 13, 12 and 3 mg of crude oils, respectively, which were treated with ethereal CH_2N_2 before analysis by GLC. Free compounds [(+)-**5** and **72-76**] were not detected in the organic layer of non-hydrolyzed-conjugate fraction.

Preparation of methyl 3'α-fluorophaseate (**73a**) *and methyl 3'β-fluorophaseate* (**74a**)

To a stirred solution of **75a** (0.1 mg) in acetone (0.5 ml) was added Jones reagent[109] (25 ml) at 5°C. The mixture was stirred for 0.5 hr at 5°C. H_2O (5 ml) was added to the mixture, and the solution was extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The residual oil was purified by chromatography on silica gel (0.8 g) with hexane-EtOAc (7:3) to afford 0.1 mg of **73a** quantitatively as a colorless oil. In the same manner, **76a** (0.1 mg) gave **74a** (0.1 mg) as a colorless oil. **74a**: UV λ_{max} (MeOH) nm (ε): 264 (18,000); EIMS *m/z* (rel. int.): 312 $[M]^+$ (28), 294 $[M - H_2O]^+$ (4), 292 (4), 280 (15), 264 (5), 251 (5), 233 (12), 208 (10), 191 (12), 177 (24), 163 (29), 154 (31), 135 (34), 125 (100); HR-EIMS: $[M]^+$ at *m/z* 312.1367 ($C_{16}H_{21}O_5F$ requires 312.1373). The ^1H and ^{19}F NMR spectral data are listed in Tables 6 and 7. The other data for **73a** are described above. The values of the optical rotation of **74a** were too small to be measured.

Gas-liquid chromatography (GLC) and TLC

GLC proceeded using 1% XE-60 (support, Chromosorb W) column (3.2 mm x 2 m) at 210°C or 1% Apiezon Grease L® (support, Shimalite Q®) column (3.2 mm x 0.5 m) at 190°C at a nitrogen flow of 60 ml min^{-1}, with an electron-capture detector. The retention times of **5a**, **75a** and **76a** in XE-60 were 5.8, 8.1, and 4.6 min, respectively and those in Apiezon Grease L® were 6.0, 9.4, and 8.0 min, respectively. The retention times of the four peaks of **72a**, **73a** and **74a** in Apiezon Grease L®, respectively, were 6.0, 7.8, 10.2 and 12.1 min. Contents of the metabolites in extracts were determined on the basis of each peak area considering the difference in the sensitivity of the electron-capture detector which was calculated from the analysis of standard samples (see Table 8). Metabolites were analyzed by silica gel TLC using Kieselgel

60 F_{254} (thickness, 0.2 mm, Merck) in a solution of hexane-EtOAc (1:1). The R_f values of **72a**, **73a**, **74a**, **75a** and **76a** were 0.50, 0.61, 0.73, 0.22 and 0.28, respectively.

GC-MS

GC-MS was conducted with 1% Apiezon Grease L® column (2.6 mm x 0.1 m) and a helium flow of 150 ml min^{-1}. The temperature was maintained at 200°C for initial 10 min, then raised from 200 to 220°C at a rate of 2°C min^{-1}. The ions observed in all the four peaks (retention time 8.2, 11.6, 14.4, and 16.4 min) of a mixture of **72a**, **73a** and **74a** at equilibrium, *m/z* (rel. int. of peaks in the ratio of 6:1:1:6, respectively): 312 $[M]^+$ (8, 8, 10, 10), 294 (3, 8, 5, 6), 280 (9, 8, 7, 7), 264 (9, 17, 17, 12), 249 (6, 8, 14, 10), 233 (12, 12, 14, 16), 221 (10, 22, 20, 16), 208 (10, 20, 14, 10), 191 (12, 25, 20, 18), 177 (17, 21, 22, 20), 163 (30, 21, 33, 22), 154 (20, 25, 33, 17), 135 (32, 32, 32, 29), 125 (100, 100, 100, 100).

Bioassays

See Chapter I.2.

Chapter II

The Role of the Hydroxyl Groups for Abscisic Acid Activity

A potential analogue with high affinity for the ABA receptor would show universally strong activity. Design of highly potent analogues with strong affinity for the ABA receptor requires precise structures for binding. This may be directly investigated by analyzing a complex of ABA and the receptor. However, no ABA-binding proteins including the receptor and carrier, have been isolated owing to their low abundance, which is a common obstacle to purifying plant proteins,[113] so the structural requirements for ABA activity are supposed only by comparing the activities of active and inactive analogues. In this chapter, the author considers the status of structure-activity investigations, describes the design of two monofluoro analogues with which to probe the role of the hydroxyl groups which have not been investigated in detail so far, and discusses the role based on the activity of the synthesized analogues.

II.1 Introduction

Receptor and Uptake Carrier

The only report on ABA-binding proteins was published by Hornberg and Weiler in 1984.[114] They demonstrated that ABA photoinductively cross-links with guard cell protoplasts in proteins located at the outward-facing plasma membrane. This suggested that ABA is detected by outward-facing plasma membrane receptors. In 1994, however, there were conflicting reports about the site perceiving ABA. When ABA was microinjected directly into guard cells, it caused stomatal closure according to Allan *et al.*[115] and Schwartz *et al.*[116] whereas Anderson *et al.*[117] found that it does not inhibit stomatal opening. To harmonize these conflicting results, it has been speculated that the effects of ABA on stomatal closure are mediated by an intracellular receptor while those on opening are mediated by an extracellular receptor.[118] In barley aleurone protoplasts, ABA microinjected into the cytoplasm had no effect on α-amylase gene expression and secretory activity, suggesting that ABA is detected at the external face of the plasma membrane.[119] At present it remains unclear whether the ABA receptor is located in the cytoplasm, at the external face of the plasma membrane, or in both. Second attempt at photoaffinity labeling the ABA receptor has not yet been reported, although in 1993, Willows and Milborrow synthesized 1-azido-ABA (**78**) as a photoaffinity probe,[18] and in 1995 Cornelussen *et al.* reported the UV-induced cross-linking of ABA through its enone chromophore to anti-ABA antibodies as models of ABA-binding proteins.[21]

Another approach to investigating the ABA receptor may be the use of anti-idiotypic antibodies as receptor probes.[120] However, Hite *et al.* doubt the feasibility of using anti-idiotypic antibodies produced through the use of 4'-substituted ABA to isolate the ABA receptor, because substitution of hydrazones for the 4'-carbonyl of ABA renders it inactive in the stomatal assay.[121]

If the ABA receptor exists inside cells, the uptake of ABA into cells by the carrier would be significant for exhibiting biological activity. It has been reported that the carrier-mediated ABA uptake by tissues[122-125] and cultured cells[126-131] occurs in addition to the non-mediated diffusive uptake of undissociated ABA which is lipid-soluble. The uptake carrier is considered to act as an ABA^-/H^+ symport

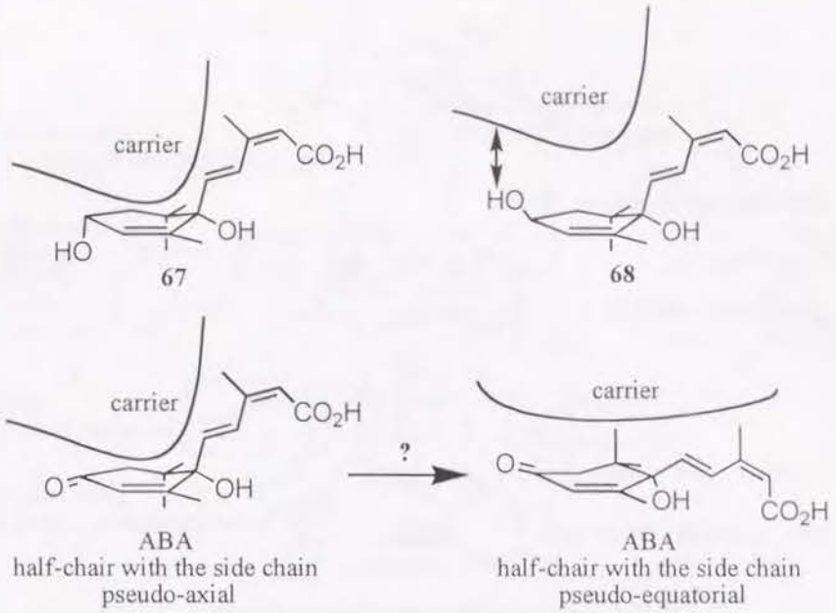
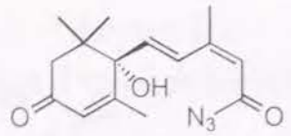


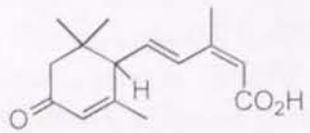
Fig .15. Binding of the diastereomeric diols (**67**, **68**) and ABA to the uptake carrier based on the Milborrow's speculation.[91]

(ABA^-: the dissociated species of ABA),[126] which is specific for (1'*S*)-(+)-ABA, K_m = *ca.* 1.0 μM. The detailed stereospecificity of the uptake has been investigated in root segments[124] and in cultured cells[127] of bean (*Phaseolus coccineus* L.) and (±)-(2*E*)-ABA (**12**), (1'*R*)-(-)-ABA and (±)-1',4'-*trans*-diol-ABA (**68**) did not reduce the uptake of (±)-[2-^{14}C]-ABA, whereas (1'*R*)-(+)-1'-deoxy-ABA (**79**), (±)-1',4'-*cis*-diol-ABA (**67**), the methyl ester of (1'*S*)-(+)-ABA (**1a**) and α- and β-ionones (**80** and **81**) specifically inhibited it. The highly inhibitory effect of **79** suggested that the 1'-hydroxyl group is not necessary for ABA to bind to the carrier. Moreover, since **67** inhibited the uptake of ABA more effectively than **68**, Milborrow and Rubery have presented the notion that the geometry of the 4'-hydroxyl group influences ligand-carrier interaction (Fig. 15).[124] This is assuming that the 4'-hydroxyl on the β-face (upper face) of the ring of **68** sterically inhibits binding to the active site on the carrier; that is, ABA interacts on the β-face of the ring with the carrier surface. This manner of binding means that the carrier surface must be convex if ABA binds to the carrier in the favored half-chair with the pseudo-axial side chain (conformation of ABA is referred later). Therefore, Milborrow speculates that ABA binds to the carrier in the disfavored half-chair with the pseudo-equatorial side chain; that is, the binding manner that allows the carrier to assume a flat surface.[91] The high affinity of **81** to the carrier supports this speculation because the side chain of **81** is frozen in the equatorial direction.[127]

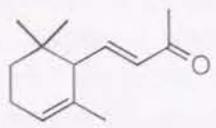
However, in carrot suspension cultured cells Windsor *et al.*[130] obtained results different from those of Milborrow and Rubery in bean root segments. The inhibitory effects of the 1',4'-diols **67** and **68** on (1'*S*)-(+)-[2-^{14}C]-ABA uptake were similar and smaller than that of (1'*S*)-(+)-ABA. Milborrow's speculation about the binding of ABA to the carrier may have a narrow application. Also, (1'*R*)-(-)-ABA competed equally with (1'*S*)-(+)-ABA at a docking site on the carrier of carrot suspension culture cells, but



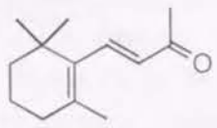
78



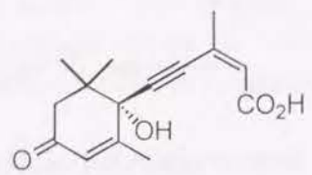
79



80



81



82

it was taken into the cells more slowly than (1'*S*)-(-)-ABA, suggesting that the carrier-mediated ABA uptake is biphasic. (2*E*)-ABA, the methyl ester of optically active ABA, PA and 4-acetylenic analogues (**82**) were less effective than (1'*S*)-(+)- and (1'*R*)-(-)-ABAs, while biologically inactive 7'-hydroxy-ABAs (**27**), biologically active (1'*S*,2'*S*)-2',3'-dihydro-ABA (**65**) and inactive (1'*S*,2'*R*)-2',3'-dihydro-ABA (**66**) were as equally effective as (1'*S*)-(+)- and (1'*R*)-(-)-ABAs, suggesting that the structural requirements for binding to the carrier differ from those for biological activity. ABA uptake into cells may be of little significance for the biological activities, suggesting that the receptor responsible for expression of the activity exists at the plasma membrane Therefore, in designing highly active analogues, affinity for the uptake carrier may be disregarded.

Structure-Activity Relationships

The activity is affected by several factors including chemical stability, permeability, affinity for the binding proteins and metabolism. The activities of many ABA analogues have been examined using different assays under various conditions,[84] so it is difficult to precisely quantify the structure-activity relationships of ABA. Considering this, the qualitative structural requirements for ABA-activity is shown in Fig. 16.[72,84,92,93,95-101,121,132-150]

Structural factors of ABA involved in the expression of the activity are (1) methyl groups at C-6, C-7', C-8' and C-9', (2) C-C double bonds at C-2, C-4 and C-2', (3) oxidized 1-carboxyl, 1'-hydroxyl and 4'-carbonyl groups, as well as the 8'-hydroxyl group introduced metabolically and (4) conformations of the six-membered ring and side chain. The absence of any one functional group reduces the activity, although to various degrees. This means that all the functional groups play a role in binding to the active site on the receptor. The methyl groups, C-6 and C-7', seem to be recognized specifically by the receptor, whereas

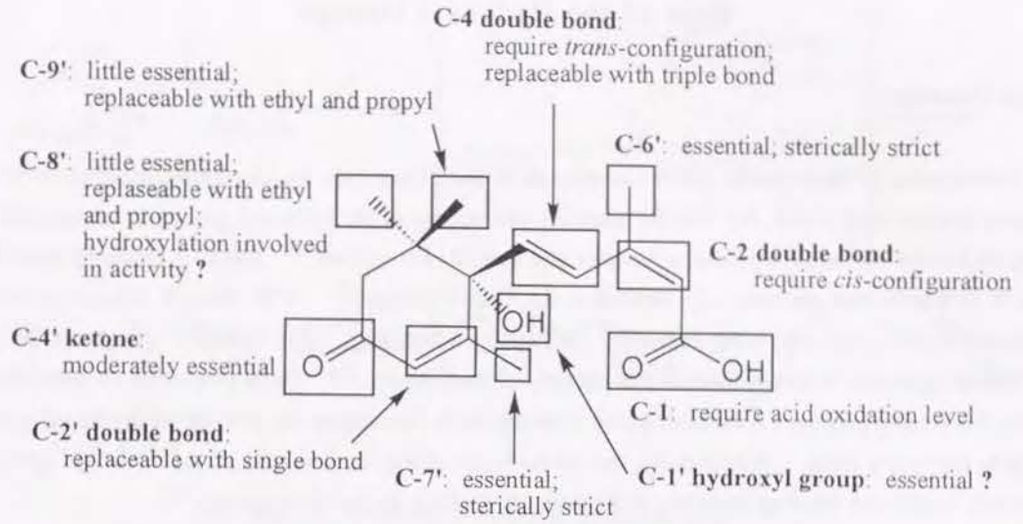


Fig. 16. Structural requirements for ABA activity supposed by the activity of known analogues.

C-8' and C-9' are considered to increase the hydrophobicity of the molecule with higher permeability to the lipid bilayer or stronger affinity to the hydrophobic region in the active site. The C-C double bonds of the side chain may give the appropriate geometry to anchor C-6 and the 1-carboxyl to the right binding site, while the 2'-double bond may fix C-7' in the right direction. The 1-carboxyl, 1'-hydroxyl and 4'-carbonyl groups are assumed to bind to polar residues of the active sites by hydrogen bonding or by other electrostatic interactions, but details of the role of the oxidized groups of ABA are unknown. The active conformation is described in detail in Chapter III.

Modifying the hydrophobic sites of the ABA molecule would have little effect on increasing affinity for the receptor, because the hydrophobic interaction cannot be strengthened without significantly increasing the steric bulkiness that sterically hinders binding to the active site. On the other hand, the hydrogen bond and electrostatic interactions through the oxidized groups of ABA can be strengthened by replacement with another electronegative atom or its introduction to the neighboring groups with an alteration in the electronegativity and dipole moment without changing the steric bulkiness. Replacement of the oxygen of ABA with another electronegative atom can not only reveal the binding manner of the oxidized groups but also increase the binding energy.

The requirement for the 1'-hydroxyl group for activity has been probed only with 1'-deoxy-ABA[132,151] which can be converted to ABA in plants,[152,153] so this remains unclear. 8'-HOABA is too unstable to examine its activity, so how the 8'-hydroxyl group is involved in the expression of the activity remains unknown, although the borate complex of 8'-HOABA is active.[56] Therefore, the author designed new analogues with which to probe the binding manner of the 1'-hydroxyl and 8'-hydroxyl groups and which may have high affinity for the binding proteins.

II.2 1'-Deoxy-1'-Fluoro- and 8'-Fluoroabscisic Acids as Probes for the Role of the Hydroxyl Groups

Design Concept

Fluorination of biologically active compounds is useful not only for designing metabolically stable analogues as described above, but also for studying interactions of the hydroxyl groups in compounds with binding molecules including a carrier, a receptor and a metabolic enzyme.[65] The C-F group of monofluoro alkane is sterically and electronically similar to the C-OH group.[154] The distinct difference between fluorine and hydroxyl groups is the capability for hydrogen bonding. The hydroxyl group can be both a donor and an acceptor, whereas fluorine can act only as an acceptor.[154] These properties of fluorine make the monofluorinated analogue a valuable probe with which to investigate the role of the hydroxyl group in binding to the active sites. Additionally, the monofluoro group as a mimic of the hydroxyl group can irreversibly bind to the binding proteins, and cause a fatal effect on the bioorganism.[65]

Thus, the author designed 1'-deoxy-1'-fluoro-ABA (**6**) and 8'-fluoro-ABA (**7**) as monofluoro analogues of ABA and 8'-HOABA which can help to identify the function of the hydroxyl groups in interactions with the binding molecules involved in the expression of the activity. The activity of **7** would also give that of 8'-HOABA. If these analogues have strong affinity for the receptor or bind irreversibly to the metabolic enzymes, they can be used as labeling compounds with which to isolate the binding proteins.

Results and Discussion

Synthesis and identification

Racemic **6** was synthesized by the direct replacement of the C-1' hydroxyl group with fluorine (Fig. 17). The methyl ester of (±)-ABA (**1a**) was fluorinated using DAST to afford compound **6a**. Saponification of **6a** with alkali resulted in several unidentified compounds, which were probably formed by the elimination of the fluorine. Hydrolysis with porcine liver esterase[155] yielded (±)-**6**. The presence of fluorine at C-1' was revealed by the spectral data. In the ^1H and ^{13}C NMR spectra, the ^1H signal of the C-5 proton and the ^{13}C signal of C-1' showed vicinal ^1H-F and ^{13}C-F coupling, respectively. The IR spectrum showed no absorption peak corresponding to the hydroxyl group at *ca* 3600 cm^{-1}.

It has been reported that the fluorinating reactions using DAST proceed through an S_N1 or S_N2 mechanism.[156-158] To examine the mechanism of this reaction in the case of the methyl ester of ABA, the ester of (*S*)-(+)-ABA was fluorinated with DAST, followed by hydrolysis. Analysis of the product by HPLC on a chiral column revealed that the *R*/*S* ratio of the product at C-1' was 1:4 (optical resolution of (±)-**6** and determination of configuration at the C-1' of each enantiomer are described below). The result indicated that the reaction proceeded mainly with the retention of the C-1' configuration, probably through the S_Ni mechanism, because the steric effect in the S_N1 mechanism, or neighboring-group participation in the S_N2 mechanism, would not occur.[159] In Fig. 17 are shown the supposed intermediate formed between the methyl ester of ABA and DAST in the S_Ni reaction.

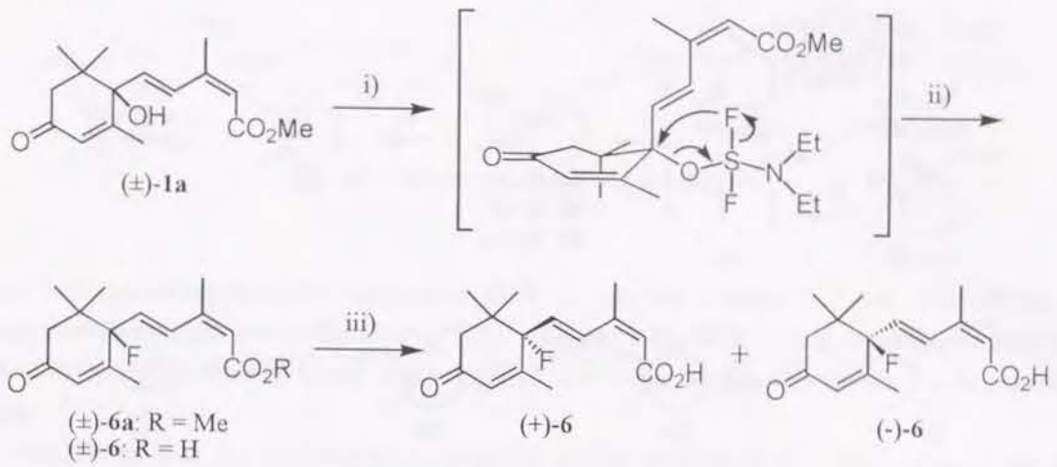


Fig. 17. Synthesis and optical resolution of **6**.
i) DAST ii) esterase iii) HPLC with Chiralcel OF

Racemic **7** was synthesized by a modification of the method reported for the synthesis of (±)-methyl phaseate[73] (Fig. 18). Each diastereomer of 2-hydroxymethyl-2,6-dimethyl-1-cyclohexanone (*trans*- and *cis*-**30**) was fluorinated using DAST. The *trans*-**30** gave the fluorinated compound *trans*-**83** in 27% yield, whereas the *cis*-**30** afforded *cis*-**83** in only 2% yield. This difference may be caused by the intramolecular hydrogen bond, which *cis*-**30** would form more easily than *trans*-**30**, because the hydroxymethyl group of *cis*-**30** located at the equatorial position is closer to the carbonyl group than that of *trans*-**30** located at the axial position. However the effect of the intramolecular hydrogen bond on the reaction remains unknown. The reaction of a mixture of *trans*- and *cis*-**83** with alkynyl lithium gave the THP ether **84**, which on deprotection to give the acetylenic diol **85**, which was then acetylated to afford the acetylenic acetate **86**. Dehydration of **86** gave the enyne acetate **87**, which was then reduced to the dienol **88**, which was oxidized to obtain the dienone **89**. Wittig reaction of **89** gave the methyl ester **90** as a mixture of (2*Z*)- and (2*E*)-isomers. Bromination of **90**, then dehydrobromination, formed the didehydro compound **91**, which on photosensitized oxygenation and subsequent adsorption with basic alumina afforded the methyl esters as an isomeric mixture. Saponification of this mixture with alkali gave 5'α,8'- and 5'β,9'-cycloabscisic acids (**10** and **11**) by the elimination of fluorine to form the cyclopropyl group. Hydrolysis with porcine liver esterase gave the free acids as an isomeric mixture of racemic **7** and its (2*E*)-isomer (**92**), and of racemic 9'-fluoroabscisic acid (**93**) and its (2*E*)-isomer (**94**) in a ratio of *ca* 1:2:2:4, as determined by HPLC on a silica gel column. This mixture was separated into its components by chromatography on silica gel and on Sephadex LH-20. The presence of fluorine at C-8' of (±)-**7** and C-9' of (±)-**93** was ascertained by the chemical shifts and the ^1H-F and ^{13}C-F couplings. In the ^1H NMR spectra, the signal of each proton of the fluoromethyl group appeared as a double doublet split by the geminal ^1H-F coupling besides the geminal ^1H-^1H coupling at δ 4.2-4.5 in both (±)-**7** and -**93**. In the ^{13}C NMR spectra, the signals of C-8' of (±)-**7** and C-9' of (±)-**92** appeared as doublets at δ 88.1 and 88.0, respectively, accord-

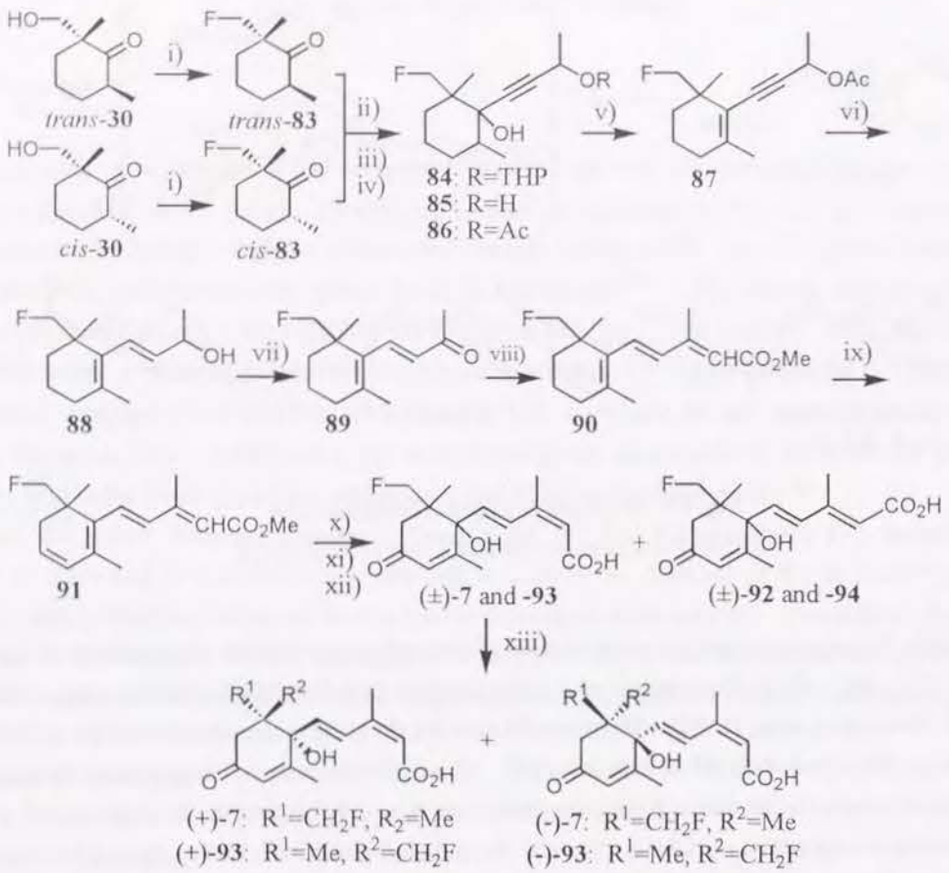


Fig. 18. Synthesis and optical resolution of 7 and **93**.

i) DAST ii) Li≡OTHP iii) pyridinium *p*-TsOH iv) Ac_2O, pyridine v) $POCl_3$, pyridine vi) Red-Al vii) MnO_2 viii) $Ph_3P{=}CHCO_2Me$ ix) a) NBS, BPO b) quinoline x) rose bengal, O_2, hv xi) basic alumina xii) esterase xiii) HPLC with Chiralcel OD.

ing to ^{13}C-F coupling. The distinction between 8'- and 9'-fluoroabscisic acids was made by the chemical shift and ^1H-F coupling of the 5-proton. When the favored conformation is the half-chair **HC1** with the side chain pseudo-axial, C-9' is spatially closer to the 5-proton than C-8', so the influence of the 9'-fluorine on the 5-proton must be greater than that of the 8'-fluorine. The signal of the 5-proton of (±)-**93** appeared at δ 6.24 as a double doublet split by ^1H-F coupling as well as vicinal coupling with the 4-proton. That of (±)-**7** was observed at δ 6.20 as a doublet split only by the vicinal coupling, indicating that the 5-proton was too far away from the fluorine for coupling. This finding showed that (±)-**7** was (±)-8'-fluoroabscisic acid and (±)-**93** was (±)-9'-fluoroabscisic acid.

As described above, this synthetic route resulted in a (±)-**7**/(±)-**93** ratio of 1:2. This diastereomeric ratio was brought about during the photosensitized oxygenation of **91**, and hence showed that the oxygenation of 6'-monofluoromethyl-didehydro compound had lower diastereoselectivity than that of 6'-

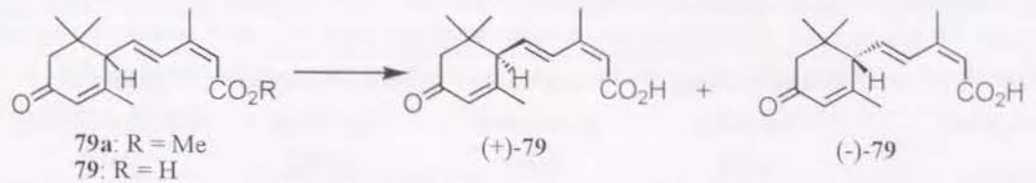


Fig. 19. Preparation of optically active 79.

di- and trifluoromethyl-didehydro compounds (8':9' = 1:4, see Chapter I.2) and that of the 6'-methoxymethyl-didehydro compound (8':9' = 1:3, see Chapter I.3). This finding meant that the hindrance effect of the monofluoromethyl group on the approach of a singlet oxygen was the smallest in these four groups.

Racemic **79** was also synthesized to compare its activity with that of **6**. This analogue has been synthesized by two groups,[132,151] but its biological activity has not been precisely examined. The methyl ester **79a** was synthesized from α-ionone (**80**)[151] (Fig. 19). Saponification of **79a** with alkali resulted in a mixture of **36** and some unidentified compounds which were formed probably through deprotonation at C-1' by the base. Therefore, the methyl ester **79a** was hydrolyzed by the esterase to afford (±)-**79**.

Racemic **6**, **79**, **7**, and **93** were optically resolved by HPLC on chiral columns to afford the (+)- and (-)-enantiomers. The Cotton effects in the CD spectra of the (+)-enantiomers of **6**, **79**, **7** and **93** were similar to those of (+)-ABA,[77] so their absolute configurations at C-1' were assumed to be the same as that of (+)-ABA. The CD spectra of the respective (-)-enantiomers were also found to be similar to that of (-)-ABA. Thus the absolute configuration at C-1' is *S* for (+)-**6**, whereas it is *R* for (+)-**79**, (+)-**7** and (+)-**93**. The notation for the configuration at C-6' for (+)-**7** is *S* and for (+)-**93** is *R*.

Biological activity

The biological activities of optically active ABA, **6**, **79**, **7**, and **93** were evaluated in the four assays described in Chapter I.2. The IC_{50} values were summarized in Table 9. (2*E*)-Isomers of racemic **6**, **7** and **93** were inactive in the assays (data not shown).

The activity of (+)-**6** was 1/10 to 1/20 that of (+)-ABA in all the assays and was almost equal to that of (+)-**79** except in the lettuce assay. This suggested that the fluorine could not act as a mimic of the hydroxyl group. If the value of 1/10-1/20 is assumed to correspond to the ratio (*K*) between the dissociation constant of (+)-ABA in binding with the receptor and that of (+)-**6** or (+)-**79**, the difference (ΔG°) in the free energy of their binding to the receptor can be estimated to be 1.4-1.8 kcal mol^{-1} at 300K (27°C), an average temperature for the assays, by using the equation $\Delta G^\circ = -RT\ln K$.[160] This implied that the contribution of the 1'-hydroxyl group to the binding energy between ABA and the receptor was about 1.4–1.8 kcal mol^{-1}, which agreed with the contribution of an uncharged hydrogen bond (0.5–1.8 kcal mol^{-1}).[161] Thus the 1'-hydroxyl group of ABA may interact with the receptor by means of an uncharged hydrogen bond. The lower activity of (+)-**6** compared to (+)-ABA suggests that the role of the 1'-hydroxyl group in this interaction is that of a hydrogen donor. Only in the lettuce assay, (+)-**79** showed relatively high activity, which was half that of (+)-ABA, whereas (+)-**6** exhibited very low activity as in the

Table 9. The IC_{50} Values for Optically Active ABA, **6**, **79**, **7** and **93** in Four Bioassays

Compound	IC_{50} in assay			
	Rice seedling elongation (μM)	Lettuce seed germination (μM)	Barley α-amylase induction (μM)	Spiderwort stomatal opening (nM)
(+)-ABA	2.0	4.2	3.1	2.6
(+)-**6**	48	50	62	33
(+)-**79**	18	9.0	35	32
(+)-**7**	2.5	4.0	9.0	3.0
(+)-**93**	1.9	8.3	9.0	3.3
(-)-ABA	2.4	12	8.9	35
(-)-**6**	50	58	80	120
(-)-**79**	18	25	68	48
(-)-**7**	2.5	13	36	24
(-)-**93**	12	12	36	38

other assays. This difference may result from the difference in the rate of permeation or uptake, or in the metabolic processing. The compound (+)-**79** might be converted more slowly than (+)-**6** to the glucose ester, which is the major metabolite in lettuce seed,[82] owing to the lack of the hydrogen acceptor at C-1'. The alternative explanation is metabolic conversion to ABA with oxidation at C-1'. However, the ability to oxidize the 1'-carbon has been different according to plants,[152,153,162] and it has not been examined whether there is the ability in lettuce seeds.

The (+)-enantiomers of **7** and **93** were as effective as, or slightly less effective than (+)-ABA in all the assays. The finding that (+)-**7**, -**2**, and -**3** (see Chapter I.2) is active equally with (+)-ABA in the stomata assay suggests that the 8'-hydroxyl group of 8'-HOABA is independent of both strengthen and weaken the activity. Therefore, 8'-HOABA is probably active as a mimic of ABA, not as an essential substance for the ABA activity. This agrees with the recent demonstration of Zou *et al.* that the biological activity of a borate complex of 8'-HOABA is comparable to that of ABA.[56] The activity of (+)-**93** suggests that the 9'-fluoro group also is little influence on exhibiting the activity.

Furthermore, the effect of (+)-**6**, -**7** and -**93** on the outward appearance of the seedlings, seeds and tissues tested were very similar to that of (+)-ABA, that is, reversible and nontoxic as far as we observed during the assays. If these compounds had bound to the receptor or metabolic enzymes then they would be expected to be more potent than (+)-ABA. Their usual or low activity and nontoxicity suggested that these analogues neither had a strong affinity for the receptor nor act as the fatal inhibitor of the monooxygenase and cyclase. The analogue (+)-**7** may be metabolized to 8'-fluoro-PA or to PA after the release of fluorine, without binding irreversibly to the catalytic site of the enzymes.

The relative intensity of the activities of the (-)-enantiomers of **6**, **79**, **7** and **93** to (-)-ABA in the four assays was similar to that of the (+)-enantiomers to (+)-ABA. This finding was consistent with the hypothesis that (+)- and (-)-ABAs bind the same receptor due to their relatively symmetrical structure.[91]

In conclusion, the present results show that the 1'-hydroxyl group as the proton donor is essential for high activity and the hydrogen bond and electrostatic interactions at C-8' is unnecessary for activity, so it may be difficult to strengthen the affinity for the active site on the receptor by modifying the 1'-hydroxyl and 8'-hydroxyl groups.

Experimental

General procedures

^1H and ^{13}C NMR spectra were recorded with TMS as the internal standard using a Jeol GX400 (400 MHz) and a Bruker ARX500 (500 MHz). For clarity, the atoms of all the compounds with the carbon skeleton of ABA are numbered as in ABA in the assignment of peaks in the ^1H and ^{13}C NMR spectra. Mass spectra were obtained with a Jeol JMS-DX300/DA5000 mass spectrometer. GC-MS was conducted with a 1% OV-17 column (1 m x 2.6 mm) in the EI mode.

(±)-1'-*Deoxy*-1'-*fluoroabscisic acid* (**6**)

DAST (120 mg) was added to a stirred solution of the methyl ester of (±)-ABA (75 mg) in Et_2O (5 ml) cooled to -78°C under N_2. The mixture was then warmed to room temperature and stirred for 3 hr. After quenching with H_2O, the mixture was extracted with Et_2O. The organic layer was washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. The residual oil was chromatographed on silica gel (2 g) with hexane-EtOAc (9:1) to give the methyl ester **6a** (43 mg, 57% yield). The methyl ester **6a** (13 mg) was dissolved in MeOH (0.8 ml) and potassium phosphate buffer (0.1 M, pH 8.0, 3 ml), and porcine liver esterase (EC 3.1.1.1, Sigma E-3128, 1270 units in 0.5 ml of 3.2 M $(NH_4)_2SO_4$, pH 8) was added. The solution was left at 30°C overnight, then diluted with H_2O (40 ml), acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (4.5 g) with hexane-EtOAc-AcOH (90:10:1) to give **6** (10 mg, 81% yield) as a colorless oil. ^1H NMR (400 MHz, CD_3OD): δ 1.07 (3H, s, H-9'), 1.13 (3H, d, $^4J_{HF}$ =1.2 Hz, H-8'), 1.94 (3H, dd, J =1.5 Hz and $^4J_{HF}$ = 2.1 Hz, H-7'), 2.04 (3H, d, J = 1.2 Hz, H-6), 2.25 (1H, ddd, J = 17.1 and 1.2 Hz, and $^4J_{HF}$ =5.2 Hz, H-5'-*pro-R*), 2.60 (1H, d, J = 17.1 Hz, H-5'-*pro-S*), 5.80 (1H, br s, H-2), 5.94 (1H, dq, J = 1.5 and 1.2 Hz, H-3'), 6.23 (1H, ddd, J = 16.2 and 0.6 Hz, and $^3J_{HF}$ = 19.2 Hz, H-5), 7.85 (1H, d, J = 16.2 Hz, H-4); ^{13}C NMR (125 MHz, acetone-d_6): δ 16.8 (d, $^3J_{CF}$ = 6.4 Hz, C-7'), 20.1 (C-6), 22.1 (d, $^3J_{CF}$ = 6.3 Hz, C-8'), 23.5 (C-9'), 40.7 (d, $^2J_{CF}$ = 21.5 Hz, C-6'), 49.0 (d, $^3J_{CF}$ = 6.2 Hz, C-5'), 99.8 (d, $^1J_{CF}$ = 184.4 Hz, C-1'), 118.9 (C-2), 127.1 (d, $^3J_{CF}$ = 5.0 Hz, C-3'), 128.5 (d, $^3J_{CF}$ = 12.1 Hz, C-4), 132.2 (d, $^2J_{CF}$ = 23.6 Hz, C-5), 149.4 (C-3), 157.5 (d, $^2J_{CF}$ = 24.0 Hz, C-2'), 166.2 (C-1), 205.3 (d, $^4J_{CF}$ = 39.2 Hz, C-4'); UV λ_{max} (MeOH) nm (ε): 238 (21,000); UV of the methyl ester λ_{max} (MeOH) nm (ε): 263.5 (26,400); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3000, 2950, 1710, 1660, 1630, 1600, 1450, 1380, 1240, 1160; EIMS (probe), *m/z* (rel. int.): 266 $[M]^+$ (1), 246 (4), 210 (4), 192 (30), 164 (100), 156 (48), 136 (21), 111 (34); HR-EIMS: $[M]^+$ at *m/z* 266.1349 ($C_{15}H_{19}O_3F$ requires 266.1319).

The reaction mechanism of DAST with (+)-ABA

A methyl ester of (+)-ABA (50 mg) which was provided by Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan was fluorinated with DAST (80 mg) and then hydrolyzed by the same method as that for (±)-**6**. The enantiomeric composition of the product 1'-deoxy-1'-fluoroabscisic acid was analyzed by HPLC on a Chiralcel OF column as well as (±)-**6** as described in the *Optical resolution*.

(±)-2-*Fluoromethyl*-2,6-*dimethyl*-1-*cyclohexanone* (*trans*- and *cis*-**83**)

Chromatography of **30** (25 g) on silica gel (970 g) with hexane-EtOAc (17:3-4:1) gave the *trans*-**30** (11.1 g) and *cis*-**30** (13.5 g). Isomer *trans*-**30** (0.25 g) in Et_2O (5 ml) was treated with DAST (0.52 g) in Et_2O (7 ml) in the same manner as that described for **6**. The product was chromatographed on silica gel (6 g) with hexane-EtOAc (24:1) to give *trans*-**83** (67 mg, 27% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 1.04 (3H, d, J = 6.4 Hz, Me-6), 1.11 (3H, d, $^4J_{HF}$ = 1.2 Hz, Me-2), 1.35-2.14 (6H, *m*, H-3, H-4, and H-5), 2.62 (1H, *m*, H-6), 4.41 (1H, dd, J = 9.2 Hz and $^2J_{HF}$ = 47.3 Hz, CH_2F), 4.58 (1H, dd, J = 9.2 Hz and $^2J_{HF}$ = 47.9 Hz, CH_2F); GC-MS 70 eV, *m/z* (rel. int.): 158 $[M]^+$ (30), 100 (20), 95 (14), 82 (43), 74 (18), 69 (100). In the same manner as *trans*-**30**, *cis*-**30** (0.5 g) gave *cis*-**83** (10.2 mg, 2% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 1.01 (3H, d, J = 6.4 Hz, Me-6), 1.21 (3H, d, $^4J_{HF}$ = 1.8 Hz, Me-2), 1.29-2.10 (6H, *m*, H-3, H-4, and H-5), 2.63 (1H, *m*, H-6), 4.30 (1H, dd, J = 9.2 Hz and $^2J_{HF}$ = 47.6 Hz, CH_2F), 4.52 (1H, dd, J = 9.2 Hz and $^2J_{HF}$ = 47.6 Hz, CH_2F); GC-MS *m/z* (rel. int.): 158 $[M]^+$ (44), 110 (24), 100 (42), 83 (32), 82 (64), 81 (40), 70 (55), 69 (100). The mixture of *trans*- and *cis*-**30** (240 g) was added dropwise to a solution of DAST (180 g) in Et_2O (500 ml) cooled to -78°C under N_2. The mixture was then warmed to room temperature and stirred for 20 hr. After quenching with H_2O, the mixture was extracted with Et_2O. The organic layer was washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. Vacuum distillation of the residual oil gave a mixture of *trans*- and *cis*-**83** (44.6 g, 18% yield) as a colorless oil, bp 80-88° (13 mmHg).

(±)-4-(1'-*Hydroxy*-2'-*fluoromethyl*-2',6'-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*-*THP* (**84**)

A 1.6 M solution of *n*-BuLi in hexane (350 ml) was added dropwise to a stirred solution of 1-methyl-2-propynyl THP ether (90 g) in THF (300 ml) over 1 hr at -78°C under N_2. After stirring for 1 hr, the reaction mixture was warmed to -25°C, and a mixture of *trans*- and *cis*-**83** (30 g) in THF (50 ml) was added dropwise. The mixture was stirred for 2 hr at -25 to -10°C, then warmed to room temperature. After quenching with 0.1 M NH_4Cl (700 ml), the mixture was extracted with Et_2O, and the organic layer was successively washed with 0.1 M NH_4Cl and H_2O, dried over Na_2SO_4, and concentrated to give a crude oil. Purification by chromatography on silica gel (1.2 kg) with hexane-EtOAc (7:3) to give **84** (49 g, 83% yield) as a mixture of diastereomers. ^1H NMR (500 MHz, $CDCl_3$) of the major diastereomer: δ 1.05 (3H, d, J = 6.7 Hz, Me-6'), 1.18 (3H, s, Me-2'), 1.22-1.90 (12H, *m*, H-3', H-4', H-5', H-2", H-3", and H-4"), 1.49 (3H, d, J = 6.7 Hz, H-1), 3.53 (2H, *m*, H-5"), 4.49 (1H, dd, J =9.2 Hz and $^2J_{HF}$ = 47.7 Hz, CH_2F), 4.70 (1H, dd, J = 9.2 Hz and $^2J_{HF}$ = 47.9 Hz, CH_2F), 4.58 (1H, *m*, H-2), 4.92 (1H, *m*, H-1"); EIMS *m/z* (rel. int.): 312 $[M]^+$ (1), 228 (3), 210 (9), 190 (10), 175 (12), 147 (13), 133 (15), 121 (26), 109 (27), 93 (29), 85 (100).

(±)-4-(1'-*Hydroxy*-2'-*fluoromethyl*-2',6'-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*. (**85**)

To a stirred solution of **84** (49 g) in EtOH (600 ml) was added pyridinium *p*-toluenesulfonate (4 g), and the mixture was stirred at 55°C for 4 hr. The solution was concentrated and the residue was diluted with Et_2O (1 litre), successively washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. Chromatography of the residual oil on silica gel (900 g) with hexane-EtOAc (7:3) gave **85** (30 g, 84% yield) as a mixture of diastereomers. ^1H NMR (500 MHz, $CDCl_3$) of the major diastereomer: δ 1.04 (3H, d, J = 6.5 Hz, Me-6'), 1.19 (3H, s, Me-2'), 1.25-1.90 (6H, *m*, H-3', H-4', and H-5'), 1.49 (3H, d, J = 6.5 Hz, H-1), 4.52 (1H, dd, J =9.2 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F), 4.62 (1H, q, J = 6.5 Hz, H-2), 4.69 (1H, dd, J = 9.2 Hz and $^2J_{HF}$ = 47.9 Hz, CH_2F); EIMS *m/z* (rel. int.): 228 $[M]^+$ (16), 196 (25), 175 (29), 148 (30), 139 (58), 121 (100), 111 (38).

(±)-3-(1'-*Hydroxy*-2'-*fluoromethyl*-2',6'-*dimethylcyclohexyl*)-1-*methyl*-2-*propynyl acetate* (**86**)

A solution of **85** (30 g) and Ac_2O (110 ml) in pyridine (200 ml) was stirred at room temperature for 15 hr. The solution was poured into ice-cooled H_2O and extracted with Et_2O. The organic layer was successively washed with 0.1 N HCl, saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel (900 g) with hexane-EtOAc (9:1) to give **86** (33 g, 93% yield) as a mixture of diastereomers. ^1H NMR (500 MHz, $CDCl_3$) of the major diastereomer: δ 1.02 (3H, d, J = 6.4 Hz, Me-6'), 1.17 (3H, s, Me-2'), 1.24-1.90 (6H, *m*, H-3', H-4', and H-5'), 1.51 (3H, d, J = 6.7 Hz, Me-1), 2.07 (3H, s, OAc), 4.49 (1H, dd, J =9.2 Hz and $^2J_{HF}$ = 47.7 Hz, CH_2F), 4.69 (1H, dd, J =9.2 Hz and $^2J_{HF}$ = 47.9 Hz, CH_2F), 5.47 (1H, q, J = 6.7 Hz, H-1); EIMS *m/z* (rel. int.): 270 $[M]^+$ (1), 208 (14), 190 (7), 175 (10), 147 (11), 121 (20), 109 (24), 91 (25), 80 (100).

(±)-3-(2'-*Fluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-1-*methyl*-2-*propynyl acetate* (**87**)

To a stirred solution of **86** (56 g) in pyridine (200 ml), a mixture of $POCl_3$ (115 ml) and pyridine (200 ml) was added dropwise at 0°C, then the solution was heated at 100°C for 3 hr. The solution was poured into ice-cooled H_2O, and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated to give a crude oil. Purification by chromatography on silica gel (330 g) with hexane-EtOAc (97:3) to give **87** (11 g, 21% yield) as a mixture of two diastereomers. ^1H NMR (500 MHz, $CDCl_3$): δ 1.08 (3/2H, d, $^4J_{HF}$ = 5.6 Hz, Me-2'), 1.09 (3/2H, d, $^4J_{HF}$ = 5.6 Hz, Me-2'), 1.37-2.06 (6H, *m*, H-3', H-4', and H-5'), 1.52 (3H, d, J = 6.6 Hz, Me-1), 1.89 (3H, s, Me-6'), 2.07 (3H, s, OAc), 4.18 (1/2H, dd, J =5.7 Hz and $^2J_{HF}$ = 47.7 Hz, CH_2F), 4.23 (1/2H, dd, J =5.7 Hz and $^2J_{HF}$ = 47.7 Hz, CH_2F), 4.37 (1/2H, dd, J =8.9 Hz and $^2J_{HF}$ = 48.3 Hz, CH_2F), 4.40 (1/2H, dd, J =8.9 Hz and $^2J_{HF}$ = 48.3 Hz, CH_2F), 5.59 (1H, q, J = 6.6 Hz, H-1); EIMS *m/z* (rel. int.): 252 $[M]^+$ (11), 208 (22), 185 (26), 175 (39), 159 (55), 137 (47), 115 (54), 105 (75), 91 (100).

(±)-(E)-4-(2'-*Fluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*ol* (**88**)

To a stirred solution of **87** (4.5 g) in THF (80 ml), a mixture of Red-Al (3.4 M in toluene, 85 ml) and THF (60 ml) was added dropwise at 0°C over 30 min under N_2. The solution was refluxed for 2 hr. Saturated NH_4Cl was added to quench the reaction, and the mixture was filtered and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated. The residual oil was chromatographed on silica gel (120 g) with hexane-EtOAc (19:1) to give **88** (3.3 g, 87% yield) as a

mixture of two diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.01 (3H, d, $^4J_{HF}$ = 2.1 Hz, Me-2'), 1.31 (3H, d, J = 6.4 Hz, H-1), 1.35-2.03 (6H, *m*, H-3', H-4', and H-5'), 1.69 (3H, s, Me-6'), 4.08 (1/2H, dd, J =5.6 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F), 4.10 (1/2H, dd, J =5.6 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F), 4.29 (1/2H, dd, J =8.9 Hz and $^2J_{HF}$ = 48.4 Hz, CH_2F), 4.29 (1/2H, dd, J =8.9 Hz and $^2J_{HF}$ = 48.4 Hz, CH_2F), 4.37 (1H, dq, J = 6.5 and 6.4 Hz, H-2), 5.50 (1H, dd, J = 15.9 and 6.5 Hz, H-3), 6.01 (1H, d, J = 15.9 Hz, H-4); EIMS *m/z* (rel. int.): 212 $[M]^+$ (4), 194 (21), 179 (30), 161 (53), 154 (24), 121 (100), 105 (34).

(±)-(E)-4-(6'-*Fluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*one* (**89**)

A mixture of active MnO_2 (30 g) and **88** (3.3 g) was stirred in CH_2Cl_2 (200 ml) at room temperature for 4 hr. The suspension was filtered, and the resulting cake of MnO_2 was washed with CH_2Cl_2. After being concentrated, the residual oil was purified by chromatography on silica gel (80 g) with hexane-EtOAc (19:1) to give **89** (3.1 g, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.09 (3H, d, $^4J_{HF}$ = 2.0 Hz, Me-6'), 1.39-2.13 (6H, *m*, H-3', H-4', and H-5'), 1.79 (3H, s, Me-2'), 2.30 (3H, s, H-1), 4.16 (1H, dd, J =9.0 Hz and $^2J_{HF}$ = 47.6 Hz, CH_2F), 4.31 (1H, dd, J =9.0 Hz and $^2J_{HF}$ = 48.1 Hz, CH_2F), 6.09 (1H, d, J = 16.4 Hz, H-3), 7.22 (1H, d, J = 16.4 Hz, H-4); EIMS *m/z* (rel. int.): 210 $[M]^+$ (5), 195 (73), 181 (11), 149 (8), 131 (17), 105 (15), 91 (21), 77 (15), 69 (100).

(±)-(2Z,4E *and* 2E,4E)-*Methyl* 5-(6'-*fluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*methyl*-2,4-*pentadienoate* (**90**)

A mixture of **89** (3.1 g) and methyl (triphenylphosphoranylidene)acetate (10.5 g) was stirred at 175°C for 2 hr, then dissolved in EtOAc (50 ml). The solution was concentrated and the residue was chromatographed on silica gel (80 g) with hexane-EtOAc (99:1) to give **90** (2.7 g, 69% yield) as a mixture of two geometrical isomers (2*Z*:2*E* = 3:7, determined by integrating the C-6' methyl singlets in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.04 (3H, d, $^4J_{HF}$ = 2.1 Hz, Me-6'-2*E*), 1.09 (3H, d, $^4J_{HF}$ = 2.0 Hz, Me-6'-2*Z*), 1.37-2.10 (12H, *m*, H-3', H-4', and H-5'), 1.72 (3H, s, Me-2'-2*E*), 1.80 (3H, s, Me-2'-2*Z*), 2.04 (3H, d, J = 1.2 Hz, H-6-2*Z*), 2.33 (3H, d, J = 1.1 Hz, H-6-2*E*), 3.69 (3H, s, CO_2Me-2*Z*), 3.71 (3H, s, CO_2Me-2*E*), 4.10 (1H, dd, J =8.9 Hz and $^2J_{HF}$ = 47.7 Hz, CH_2F-2*E*), 4.15 (1H, dd, J =8.9 Hz and $^2J_{HF}$ = 47.7 Hz, CH_2F-2*Z*), 4.29 (1H, dd, J =8.9 Hz and $^2J_{HF}$ = 48.3 Hz, CH_2F-2*E*), 4.35 (1H, dd, J =8.9 Hz and $^2J_{HF}$ = 48.4 Hz, CH_2F-2*Z*), 5.67 (1H, s, H-2-2*Z*), 5.75 (1H, s, H-2-2*E*), 6.08 (1H, d, J = 16.1 Hz, H-4-2*E*), 6.50 (1H, d, J = 16.1 Hz, H-5-2*E*), 6.53 (1H, d, J = 16.4 Hz, H-5-2*Z*), 7.61 (1H, d, J = 16.4 Hz, H-4-2*Z*); EIMS *m/z* (rel. int.): 266 $[M]^+$ (56), 251 (7), 234 (17), 219 (4), 207 (18), 199 (21), 187 (12), 178 (38), 159 (45), 145 (34), 133 (47), 125 (82), 119 (100), 112 (30), 105 (36).

(±)-(2Z,4E *and* 2E,4E)-*methyl* 5-(6'-*fluoromethyl*-2',6'-*dimethyl*-3',4'-*didehydro*-1'-*cyclohexene*-1'-*yl*)-3-*methyl*-2,4-*pentadienoate* (**91**)

NBS (4.2 g) and BPO (40 mg) were added to a solution of **90** (3.7 g) in CCl_4 (40 ml), and the mixture was then refluxed for 2 hr under N_2. After cooling the mixture to room temperature, it was filtered, and quinoline (13.5 ml) was added to the filtrate. The mixture was concentrated, and the residue

was heated at 100°C for 1 hr under N_2. After cooling to room temperature, the reaction mixture was poured into 1% H_2SO_4 (400 ml) and extracted with Et_2O. The organic layer was successively washed with saturated $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. The residual oil was purified by chromatography on silica gel (60 g) with hexane-EtOAc (39:1) to give the didehydro compound **91** (2.0 g, 55% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.11 (3H, d, $^4J_{HF}$ = 1.5 Hz, Me-6'-2*E*), 1.15 (3H, d, $^4J_{HF}$ = 1.5 Hz, Me-6'-2*Z*), 1.87 (3H, s, Me-2'-2*E*), 1.95 (3H, s, Me-2'-2*Z*), 2.06 (3H, d, J = 1.1 Hz, H-6-2*Z*), 2.26-2.42 (4H, m, H-5'), 2.34 (3H, d, J = 1.0 Hz, H-6-2*E*), 3.70 (3H, s, CO_2Me-2*Z*), 3.72 (3H, s, CO_2Me-2*E*), 4.14 (1H, dd, J =8.8 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F-2*E*), 4.17 (1H, dd, J =8.7 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F-2*Z*), 4.32 (1H, dd, J =8.8 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F-2*E*), 4.35 (1H, dd, J =8.7 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F-2*Z*), 5.69 (1H, s, H-2-2*Z*), 5.78 (1H, s, H-2-2*E*), 5.79-5.90 (4H, m, H-3' and H-4'), 6.21 (1H, d, J = 16.2 Hz, H-4-2*E*), 6.52 (1H, d, J = 16.2 Hz, H-5-2*E*), 6.55 (1H, d, J = 16.5 Hz, H-5-2*Z*), 7.76 (1H, d, J = 16.5 Hz, H-4-2*Z*); EIMS *m/z* (rel. int.): 264 $[M]^+$ (63), 249 (17), 231 (15), 205 (42), 199 (100), 185 (22), 171 (56), 157 (52), 143 (32), 119 (56).

(±)-8'-*Fluoroabscisic acid* (**7**), *its* (2E)-*isomer* (**92**), (±)-9'-*fluoroabscisic acid* (**93**), *and its* (2E)-*isomer* (**94**)

A solution of **91** (2.0 g) and rose bengal (0.35 g) in MeOH (250 ml) was stirred under O_2 under fluorescent irradiation at 30°C for 12 hr. After being concentrated, the residue was dissolved in MeOH (20 ml), and alumina (active basic, 15 g) was added to the solution. After evaporating the MeOH, hexane (15 ml) was added to the mixture, and the suspension was stirred at room temperature for 2 hr before being chromatographed on alumina (80 g). Elution with 10-100% EtOAc in hexane afforded the crude ester as an oil. The crude ester was purified by chromatography on silica gel (40 g) with hexane-EtOAc (4:1) to give 790 mg (35% yield) of a mixture of four isomers. This mixture (300 mg) was dissolved in MeOH (5 ml) and potassium phosphate buffer (0.1 M, pH 8.0, 25 ml), and porcine liver esterase (12400 units in 4.9 ml) was added. The solution was left at 30° over night, then diluted with H_2O (200 ml), acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (30 g) with 4% AcOH in CH_2Cl_2 to give 106 mg of a mixture of (2*E*)-isomers and 115 mg of a mixture of (2*Z*)-isomers. The (2*Z*)-isomers (70 mg) were separated again by chromatography on silica gel (30 g) with 1% TFA in CH_2Cl_2 to give 42 mg of (±)-**7** and 22 mg of (±)-**93** as white amorphous powders. (2*E*)-Isomers (100 mg) were separated by chromatography on Sephadex LH-20 (25 g) with 4% AcOH in CH_2Cl_2 to give 6.1 mg of (±)-**92** as a white amorphous powder, 8.4 mg of (±)-**94** as a white amorphous powder. (±)-**7**. ^{1}H NMR (500 MHz, CD_3OD): δ 1.07 (3H, d, $^4J_{HF}$ = 1.4 Hz, H-9'), 1.92 (3H, d, J = 1.3 Hz, H-7'), 2.05 (3H, d, J = 0.8 Hz, H-6), 2.44 (1H, dd, J = 17.4 and 1.4 Hz, H-5'-*pro-R*), 2.51 (1H, d, J = 17.4 Hz, H-5'-*pro-S*), 4.38 (1H, dd, J =9.4 Hz and $^2J_{HF}$ = 48.0 Hz, CH_2F), 4.55 (1H, dd, J = 9.4 Hz and $^2J_{HF}$ = 48.2 Hz, CH_2F), 5.78 (1H, br s, H-2), 5.97 (1H, br s, H-3'), 6.20 (1H, d, J = 16.2 Hz, H-5), 7.76 (1H, d, J = 16.2 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD): δ 19.1 (d, $^3J_{CF}$ = 4.3 Hz, C-9'), 19.4 (C-7'), 21.2 (C-6), 44.4 (d, $^3J_{CF}$ = 5.1 Hz, C-5'), 46.9 (d, $^2J_{CF}$ = 15.5 Hz, C-6'), 79.2 (C-1'), 88.1 (d, $^1J_{CF}$ = 170.8 Hz, C-8'), 120.1 (C-2), 128.3 (C-3'), 129.8 (C-4), 136.9 (C-5), 150.6 (C-3), 165.7 (C-2'), 169.6 (C-1), 199.6 (C-4'); UV λ_{max} (MeOH) nm (ε): 244 (19,100); UV of the methyl ester λ_{max} (MeOH) nm (ε): 264

(19,300); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3560, 3000, 2950, 1700, 1660, 1630, 1600, 1430, 1375, 1235, 1160; EIMS *m/z* (rel. int.): 282 $[M]^+$ (4), 264 (10), 231 (11), 223 (22), 203 (7), 190 (100), 172 (22), 162 (54), 147 (16), 134 (60), 119 (15), 111 (49); HR-EIMS: $[M]^+$ at *m/z* 282.1255 ($C_{15}H_{19}O_4F$ requires 282.1268). (±)-**93**. ^1H NMR (500 MHz, CD_3OD): δ 1.06 (3H, d, $^4J_{HF}$ = 2.1 Hz, H-8'), 1.93 (3H, d, *J* = 1.3 Hz, H-7'), 2.02 (3H, d, *J* = 1.0 Hz, H-6), 2.27 (1H, d, *J* = 16.9 Hz, H-5'-*pro-R*), 2.75 (1H, d, *J* = 16.9 Hz, H-5'-*pro-S*), 4.22 (1H, dd, *J* =9.5 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F), 4.53 (1H, dd, *J* = 9.5 Hz and $^2J_{HF}$ = 48.0 Hz, CH_2F), 5.74 (1H, br s, H-2), 5.96 (1H, br s, H-3'), 6.24 (1H, dd, *J* = 16.0 Hz and $^5J_{HF}$ = 3.3 Hz, H-5), 7.75 (1H, d, *J* = 16.0 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD): δ 17.9 (d, $^3J_{CF}$ = 6.1 Hz, C-8'), 19.1 (C-7'), 21.2 (C-6), 44.9 (d, $^3J_{CF}$ = 4.0 Hz, C-5'), 47.3 (d, $^2J_{CF}$ = 15.3 Hz, C-6'), 79.1 (C-1'), 88.0 (d, $^1J_{CF}$ = 173.0 Hz, C-9'), 119.5 (C-2), 127.5 (C-3'), 129.0 (C-4), 137.2 (C-5), 151.2 (C-3), 165.4 (C-2'), 169.4 (C-1), 199.9 (C-4'); UV λ_{max} (MeOH) nm (ε): 244 (20,800); UV of the methyl ester λ_{max} (MeOH) nm (ε): 264 (19,200); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3560, 3000, 2950, 1700, 1660, 1630, 1600, 1430, 1375, 1235, 1160; EIMS *m/z* (rel. int.): 282 $[M]^+$ (3), 264 (13), 231 (10), 223 (20), 203 (6), 190 (100), 172 (12), 162 (58), 147 (17), 134 (64), 119 (16), 111 (60); HR-EIMS: $[M]^+$ at *m/z* 282.1263 ($C_{15}H_{19}O_4F$ requires 282.1268). (±)-**92**. ^1H NMR (500 MHz, CD_3OD): δ 1.06 (3H, d, $^4J_{HF}$ = 1.4 Hz, H-9'), 1.90 (3H, d, *J* = 1.3 Hz, H-7'), 2.27 (3H, d, *J* = 0.7 Hz, H-6), 2.47 (1H, dd, *J* = 17.4 and 1.3 Hz, H-5'-*pro-R*), 2.54 (1H, d, *J* = 17.4 Hz, H-5'-*pro-S*), 4.37 (1H, dd, *J* =9.3 Hz and $^2J_{HF}$ = 48.0 Hz, CH_2F), 4.54 (1H, dd, *J* = 9.3 Hz and $^2J_{HF}$ = 48.1 Hz, CH_2F), 5.86 (1H, br s, H-2), 5.95 (1H, br s, H-3'), 6.22 (1H, d, *J* = 15.7 Hz, H-5), 6.45 (1H, d, *J* = 15.7 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 246 (22,100); UV of the methyl ester λ_{max} (MeOH) nm (ε): 264 (23,100); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3560, 3000, 2950, 1705, 1660, 1630, 1610, 1430, 1350, 1230, 1160; EIMS *m/z* (rel. int.): 282 $[M]^+$ (4), 264 (7), 231 (10), 223 (18), 208 (7), 190 (100), 172 (12), 162 (48), 147 (14), 134 (61), 119 (14), 111 (27); HR-EIMS: $[M]^+$ at *m/z* 282.1266 ($C_{15}H_{19}O_4F$ requires 282.1268). (±)-**94**. ^1H NMR (500 MHz, CD_3OD): δ 1.06 (3H, d, $^4J_{HF}$ = 2.0 Hz, H-8'), 1.90 (3H, d, *J* = 1.2 Hz, H-7'), 2.25 (3H, d, *J* = 1.1 Hz, H-6), 2.30 (1H, dd, *J* = 17.1 and 0.7 Hz, H-5'-*pro-R*), 2.77 (1H, d, *J* = 17.1 Hz, H-5'-*pro-S*), 4.21 (1H, dd, *J* =9.5 Hz and $^2J_{HF}$ = 47.8 Hz, CH_2F), 4.49 (1H, dd, *J* = 9.5 Hz and $^2J_{HF}$ = 48.0 Hz, CH_2F), 5.83 (1H, br s, H-2), 5.94 (1H, br s, H-3'), 6.27 (1H, dd, *J* = 15.7 Hz and $^5J_{HF}$ = 2.9 Hz, H-5), 6.45 (1H, d, *J* = 15.7 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 246 (23,500); UV of the methyl ester λ_{max} (MeOH) nm (ε): 264 (21,800); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3560, 3000, 2950, 1705, 1660, 1630, 1610, 1430, 1360, 1230, 1160; EIMS *m/z* (rel. int.): 282 $[M]^+$ (5), 264 (8), 231 (9), 223 (18), 208 (7), 190 (100), 172 (14), 162 (41), 147 (12), 134 (59), 119 (12), 111 (25); HR-EIMS: $[M]^+$ at *m/z* 282.1265 ($C_{15}H_{19}O_4F$ requires 282.1268).

(±)-*1'-Deoxyabscisic acid* (**79**) *and its* (2E)-*isomer* (**95**)

The mixture of (±)-methyl 1'-deoxyabscisate (**79a**) and its (2*E*)-isomer (**95a**) was prepared as reported by Roberts *et al.*[151] This mixture (300 mg) was hydrolyzed by porcine liver esterase (2530 units in 1 ml) in the same manner as that of (±)-**6**. The hydrolysate was chromatographed on silica gel (5.6 g) with 10-25% EtOAc in hexane containing 1% AcOH to give a mixture of (±)-**79** and -**95** (20 mg, 35% yield). The mixture (20 mg) was separated by HPLC on a silica gel column (YMC A023, 250 x 10 mm;

solvent, 1% AcOH in $CHCl_3$; flow rate, 3.0 ml min^{-1}; detection, 254 nm) to give 10.4 mg of (±)-**79** and 7.5 mg of (±)-**95** as colorless oils. (±)-**79**. ^1H NMR (500 MHz, $CDCl_3$): δ 0.99 (3H, s, H-9'), 1.07 (3H, s, H-8'), 1.93 (3H, d, *J* =0.7 Hz, H-7'), 2.04 (3H, d, *J* = 0.7 Hz, H-6), 2.16 (1H, d, *J* = 16.9 Hz, H-5'), 2.38 (1H, d, *J* = 16.9 Hz, H-5'), 2.75 (1H, d, *J* = 9.6 Hz, H-1'), 5.74 (1H, s, H-2), 5.95 (1H, s, H-3'), 5.98 (1H, dd, *J* = 15.7 and 9.6 Hz, H-5), 7.70 (1H, d, *J* = 15.7 Hz, H-4); UV λ_{max} (MeOH) nm (loge): 264 (21,400); UV of the methyl ester λ_{max} (MeOH) nm (loge): 266 (18,600); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3000, 2950, 1710, 1660, 1625, 1610, 1430; EIMS of the methyl ester *m/z* (rel. int.): 262 $[M]^+$ (2), 247 (1), 231 (7), 215 (4), 189 (4), 174 (40), 146 (100), 125 (>100), 119 (24), 112 (19); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 262.1559 ($C_{16}H_{22}O_3$ requires 262.1569). (±)-**95**. ^1H NMR (500 MHz, $CDCl_3$): δ 0.98 (3H, s, H-9'), 1.07 (3H, s, H-8'), 1.90 (3H, d, *J* =0.9 Hz, H-7'), 2.14 (1H, d, *J* = 16.8 Hz, H-5'), 2.29 (3H, s, H-6), 2.36 (1H, d, *J* = 16.8 Hz, H-5'), 2.67 (1H, d, *J* = 9.5 Hz, H-1'), 5.81 (1H, s, H-2), 5.96 (1H, s, H-3'), 6.02 (1H, dd, *J* = 15.5 and 9.4 Hz, H-5), 6.25 (1H, d, *J* = 15.5 Hz, H-4); UV λ_{max} (MeOH) nm (ε): 257 (29,500); UV of the methyl ester λ_{max} (MeOH) nm (ε): 266 (28,200); IR of the methyl ester ν_{max} ($CHCl_3$) cm^{-1}: 3000, 2950, 1710, 1660, 1625, 1610, 1430; EIMS of the methyl ester *m/z* (rel. int.): 262 $[M]^+$ (3), 231 (7), 206 (14), 189 (4), 174 (63), 146 (100), 125 (93), 119 (21), 112 (15); HR-EIMS of the methyl ester: $[M]^+$ at *m/z* 262.1571 ($C_{16}H_{22}O_3$ requires 262.1569).

Optical resolution

When the solutions collected from the columns in this section were concentrated, toluene was added to evaporate TFA as an azeatrope. Racemic **6** (7 mg) was injected into a Chiralcel OF HPLC column (250 x 10 mm, Daicel; solvent, 8% isopropanol in hexane containing 0.1% TFA; flow rate, 3.5 ml min^{-1}; detection, 254 nm). The materials at t_R 16.4 and 18.2 min were collected to give (-)- and (+)-**6** (1.8 and 3.2 mg) with an optical purity of 99.9 and 99.2%, respectively, measured by HPLC on the same column. (-)-**6**: $[\alpha]_D^{15}$ -358° (MeOH; *c* 0.379); CD λ_{ext} (MeOH) nm (Δε): 223 (+13.3), 257 (-18.2), 319 (+1.8). (+)-**6**: $[\alpha]_D^{15}$ +357° (MeOH; *c* 0.212); CD λ_{ext} (MeOH) nm (Δε): 222 (-17.2), 254 (+19.5), 315 (-1.6). Racemic **7** (21 mg) was injected into a Chiralcel OD HPLC column (250 x 4.6 mm, Daicel; solvent, 11% isopropanol in hexane containing 0.1% TFA; flow rate, 1.2 ml min^{-1}; detection, 254 nm). The materials at t_R 8.0 and 15.0 min were collected to give (+)- and (-)-**7** (10.2 and 10.3 mg) with an optical purity of 99.9 and 99.3%, respectively, measured by HPLC on the same column. Racemic **93** (15 mg) was injected into a Chiralcel OD column under the same conditions as described for (±)-**7**, and the materials at t_R 8.0 and 16.0 min were collected to give (+)- and (-)-**93** (7.4 and 7.4 mg) with optical purity of 99.9 and 99.5%, respectively, measured by HPLC on the same column. (+)-**7**: $[\alpha]_D^{28}$ +351° (MeOH; *c* 0.667); CD λ_{ext} (MeOH) nm (Δε): 229 (-26.8), 260 (+33.5), 319 (-3.7). (-)-**7**: $[\alpha]_D^{28}$ -345° (MeOH; *c* 0.667); CD λ_{ext} (MeOH) nm (Δε): 229 (+27.5), 260 (-34.3), 319 (+2.5). (+)-**93**: $[\alpha]_D^{28}$ +368° (MeOH; *c* 0.493); CD λ_{ext} (MeOH) nm (Δε): 230 (-27.8), 261 (+34.5), 323 (-2.9). (-)-**93**: $[\alpha]_D^{28}$ -375° (MeOH; *c* 0.493); CD λ_{ext} (MeOH) nm (Δε): 228 (+28.1), 260 (-35.0), 322 (+2.9). Racemic **79** (9.4 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 t_R 11.4 and 14.4 min were collected to give (+)- and (-)-**79** (4.8 and 2.0 mg) with an optical purity of 93 and 97%, respectively, measured by HPLC

on the same column. (+)-**79**: $[\alpha]_D^{29}$ +344° (MeOH; *c* 0.2); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 216 (-11.4), 265 (+23.7), 313 (-2.3). (-)-**79**: $[\alpha]_D^{29}$ -334° (MeOH; *c* 0.2); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 216 (+11.7), 264 (-24.2), 316 (+2.4).

Bioassays

See Chapter I.2.

Chapter III
The Active Conformation of Abscisic Acid

As described in Chapter II.1, conformation is one significant factor in the structure-activity relationships of ABA. The analogues that adopt the active conformation of ABA would exhibit high activity. However, conformational requirement for activity is unknown and analogue probes have not been reported in detail. This chapter describes investigation of the active conformation of ABA using new analogues as probes.

III.1 Introduction

Ring Conformation of Abscisic Acid

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[163] in Fig. 20.

In the crystal (Fig. 1),[164,165] 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[76,166] and CD[77,167] analyses is the half-chair $\mathbf{HC_1}$ with the pseudo-axial side chain. The negative Cotton effect derived from the n-π^* transition of the enone at 320 nm in the CD spectrum results from distortion of the enone,[168-171] and the value of $\Delta\varepsilon$ (-2.34)[77] indicates that the torsion angle of C-3'-C-4' bond is about 10-20°,[169] which almost equals the torsion angle (15°) of the most stable half-chair form of cyclohexenone.[172] This suggests that the favored conformation in solution is not the sofa $\mathbf{S_1}$ but the half-chair $\mathbf{HC_1}$. 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 $\mathbf{HC_1}$ and its inverted form $\mathbf{HC_2}$ with the pseudo-equatorial side chain.

According to computer-aided conformational analysis,[166] the energy difference between $\mathbf{HC_1}$ and $\mathbf{HC_2}$ is 3 kcal mol^{-1}, meaning that the $\mathbf{HC_1}$/$\mathbf{HC_2}$ ratio in conformational equilibrium at 300 K is about 99.4:0.6 from the Gibbs equation, $\Delta G^\circ = -RT\ln K$.[173] 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,[166] 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.[174] ABA has not been analyzed by low temperature NMR.[175]

Other forms, the sofas $\mathbf{S_{1\text{-}4}}$, 1,3-diplanars $\mathbf{DP_{1\text{-}4}}$ and boats $\mathbf{B_{1\text{-}2}}$, 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.

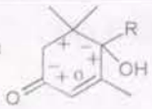
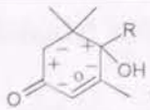
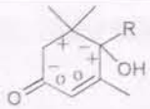
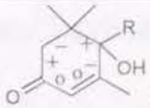
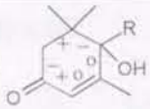
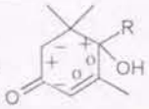
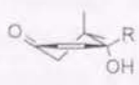
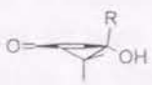
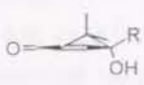
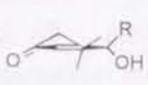
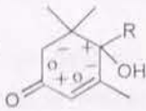
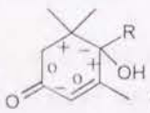
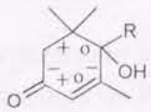
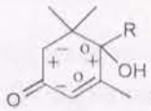
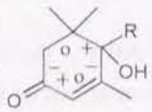
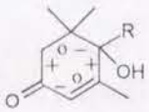
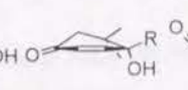
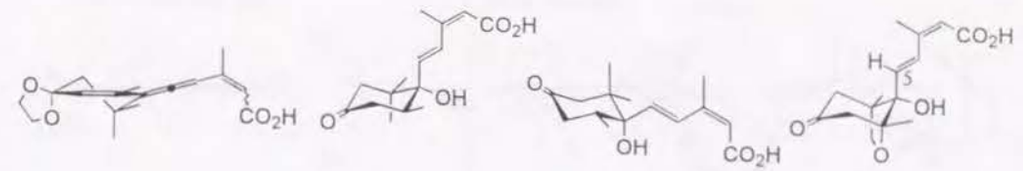
sign of torsion angle*						
side-view†						
name	half-chair **HC_1**	half-chair **HC_2**	sofa **S_1**	sofa **S_2**	sofa **S_3**	sofa **S_4**
side-chain orientation‡	pseudo-axial	pseudo-equatorial	pseudo-axial	pseudo-equatorial	bisectional	bisectional
C-9' orientation‡	equatorial	axial	equatorial	axial	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. 20. 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.

Ring Conformation-Activity Relationships

The allenic analogue (**96**) has been designed and synthesized by Abrams and Milborrow[176] based on Milborrow's speculation that ABA adopts the less favored half-chair **HC_2** in binding to the uptake carrier (Fig. 21).[91] 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, **96** with the side chain equatorial was inactive although it showed activity after conversion to ABA.[177] Churchill *et al.* reported that the (1'*S*,2'*S*)-2',3'-dihydro-ABA (**65**) is active,



96 **65** **66** **17** (PA)

Fig. 21. Steric structures of **96**, **65**, **66** and **17**.

whereas (1'*S*,2'*R*)-2',3'-dihydro-ABA (**66**) is inactive.[98] There were neither the conformational analyses of these analogues nor a discussion based on their conformations in that paper, but the two dihydro-ABAs **65** and **66** would adopt a chair form 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, PA seems to be contrary to this suggestion because PA is inactive in almost all assays[84] 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.

Other analogues that yield significant understanding of the conformational requirement for activity have not been reported, and a serious approach to probe it has not yet been taken. The author investigated the active conformation of ABA with new cyclopropane analogues having a unique property.

III.2 The Cyclopropane Analogues as Probes for the Active Conformation

Design Concept

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 **8**-**11** (Fig. 22).

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,[178,179] whereas its steric size is larger than that of olefins. In bicyclo[4.1.0]heptan-2-one, the cyclopropyl ring is constrained essentially in an axial-like orientation to the plane of the cyclohexanone ring.[180,181] Therefore, replacing the 2'-double bond of ABA with cyclopropane (**8** and **9**) 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 **8** can prefer the half-chair with the pseudo-equatorial side chain similar to the disfavored conformer **HC_2** of ABA, while **9** can prefer that with the side chain pseudo-axial similar to the favored conformer **HC_1** (Fig. 22). Analogues **10** and **11**

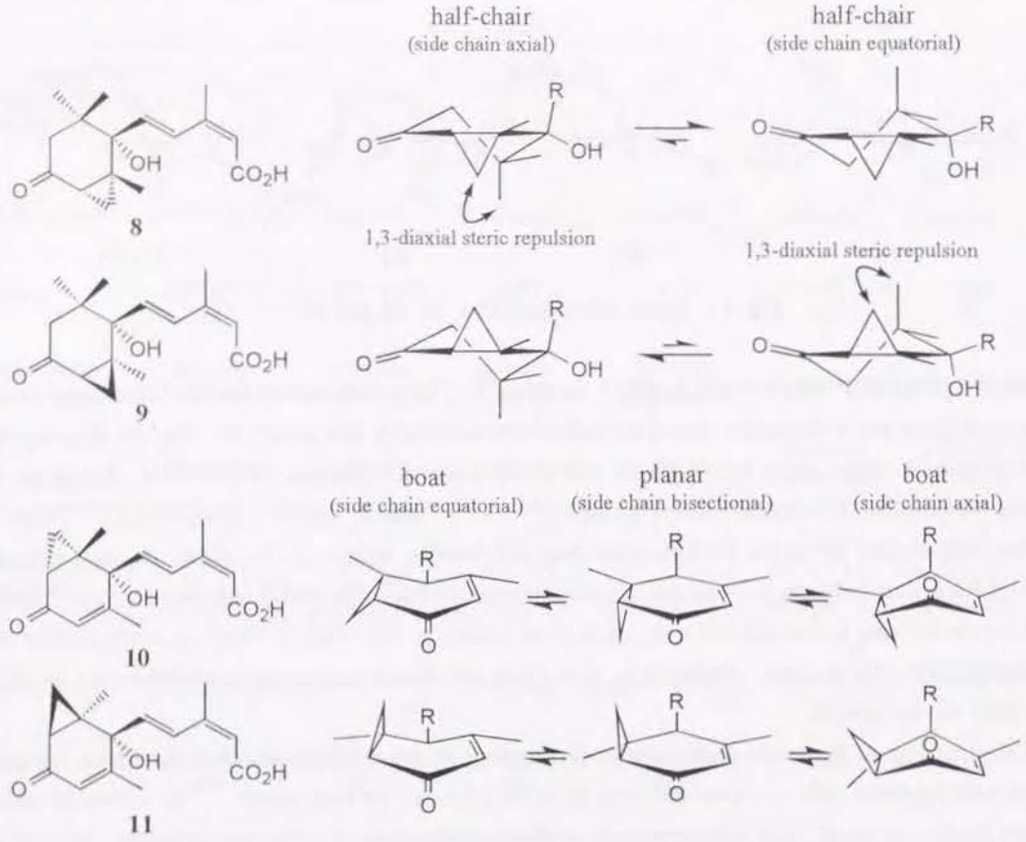


Fig. 22. Possible ring conformations of **8-11** (R: side chain).

where the C-5'-C-6' single bond is replaced with cyclopropane can adopt a cyclohexadienone-like planar or boat conformation.[182-184] The boat-boat inversion potential of 1,4-cyclohexadiene is shallow,[183] so **10** and **11** can convert the orientation of the side chain into that of the active form of ABA without a great loss of energy. In contrast to the free orientation of the side chain, orientations of the 6'-substituents can be restricted owing to the cyclopropyl ring. Compound **10** always possesses the axial-like 6'α- and equatorial-like 6'β-substituents, while **11** always possesses the equatorial-like 6'α- and axial-like 6'β-substituents (Fig. 22). Therefore, a comparison between the activities of **10** and **11** 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.

Results and Discussion

Synthesis

Racemic **8** and **9** were synthesized from the oxoisophorone ethylene ketal **97** (Fig. 23). Compound **97** was treated with trimethyloxosulfonium iodide and sodium hydride to give the cyclopropanoid **97**.[185]

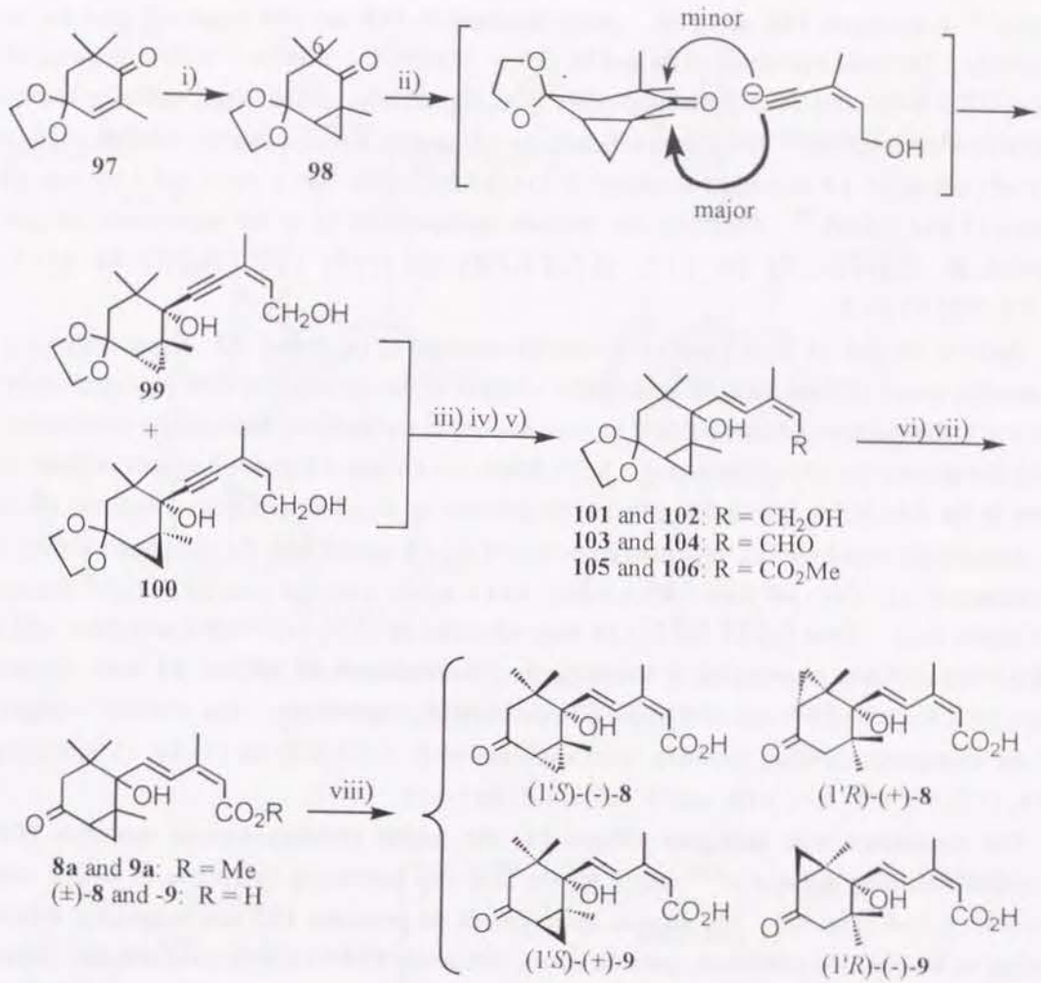
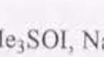


Fig. 23. Synthesis and optical resolution of **8** and **9**.

i) Me_3SOI, NaH ii) *n*-BuLi,  iii) Red-Al® iv) MnO_2
v) MnO_2, NaCN, AcOH, MeOH vi) HCl vii) NaOH viii) HPLC with Chiralcel OD.

The following synthetic route was as described by Mayer *et al.*[186] Coupling reaction of alkynyl lithium and **98** 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 (±)-**8** and -**9** described later; the minor diastereomer was **99** where the cyclopropane is *trans* to the side chain, and the major diastereomer was **100** where the cyclopropane is *cis* to the side chain. The favored conformation of **98** 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 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 **100**. Reduction of a mixture of **99** and **100** gave

a mixture of **101** and **102**, which was oxidized to a mixture of **103** and **104**, followed by a Corey oxidation[187] to the esters **105** and **106**. Acidic treatment of **105** and **106** separately gave **8a** and **9a**, respectively. The basic hydrolysis of **8a** and **9a** yielded (±)-2'α,3'α-dihydro-2'α,3'α-methano-ABA (**8**) and (±)-2'β,3'β-dihydro-2'β,3'β-methano-ABA (**9**), respectively, which were optically resolved by HPLC with a chiral column. The Cotton effects in the CD spectra revealed that the absolute configuration at C-1' of (-)-**8** and (+)-**9** was equal to natural (1'*S*)-(+)-ABA, while that of (+)-**8** and (-)-**9** was equal to unnatural (1'*R*)-(-)-ABA.[77] Therefore, the absolute configurations of all the asymmetric carbons were elucidated as (1'*S*,2'*R*,3'*S*) for (-)-**8**, (1'*S*,2'*S*,3'*R*) for (+)-**9**, (1'*R*,2'*R*,3'*S*) for (+)-**8**, and (1'*R*,2'*S*,3'*R*) for (-)-**9**.

Racemic **10** and **11** were prepared by alkaline treatment of (±)-**7** and -**93**, respectively (Fig. 24). 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, (±)-**10** and -**11** showed signals of three coupled protons in the field higher than δ 2.0, proving the presence of a cyclopropyl ring. Racemic **10** and **11** were optically resolved by HPLC on a chiral column and (+)-**10** agreed with the compound yielded by the basic treatment of (1'*R*)-(+)-8'-fluoro-ABA, while (+)-**11** agreed with that from (1'*R*)-(+)-9'-fluoro-ABA (see Chapter II.2). Thus (+)-**10** and (+)-**11** were identified as (1'*S*)-(+)-5'α,8'-cyclo-ABA and (1'*S*)-(+)-5'β,9'-cyclo-ABA, respectively. Similarly, the (-)-enantiomers of **10** and **11** 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 (+)-**10**, (1'*S*,5'*S*,6'*R*) for (+)-**11**, (1'*R*,5'*R*,6'*S*) for (-)-**10**, and (1'*R*,5'*S*,6'*R*) for (-)-**11**.

For comparison with analogues **10** and **11**, the achiral cyclohexadienone analogue **109** was synthesized according to Lei *et al*[149] except for the final step containing hydrolysis of methyl ester and deketalation at C-4' (Fig. 25). The alkaline hydrolysis of the precursor **107** and subsequent deketalation according to the reported procedure, gave the epoxy compound **108** in a 34% yield and the desired free acid **109** in an extremely low yield (6 %), which was 1/10 of the reported yield (63%). The epoxide **108** 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 **107** followed by hydrolysis with esterase in phosphate buffer at pH 8 instead of in alkali, gave **109** in a 40% yield from **107**. It has been reported that **109** is very sensitive to dilute base and so unstable in solution that its biological activity could not be tested.[149] In our experiments, although **109** 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 **109** 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 **109** was confirmed here for the first time. We cannot explain why our results differed from those of Lei *et al.*

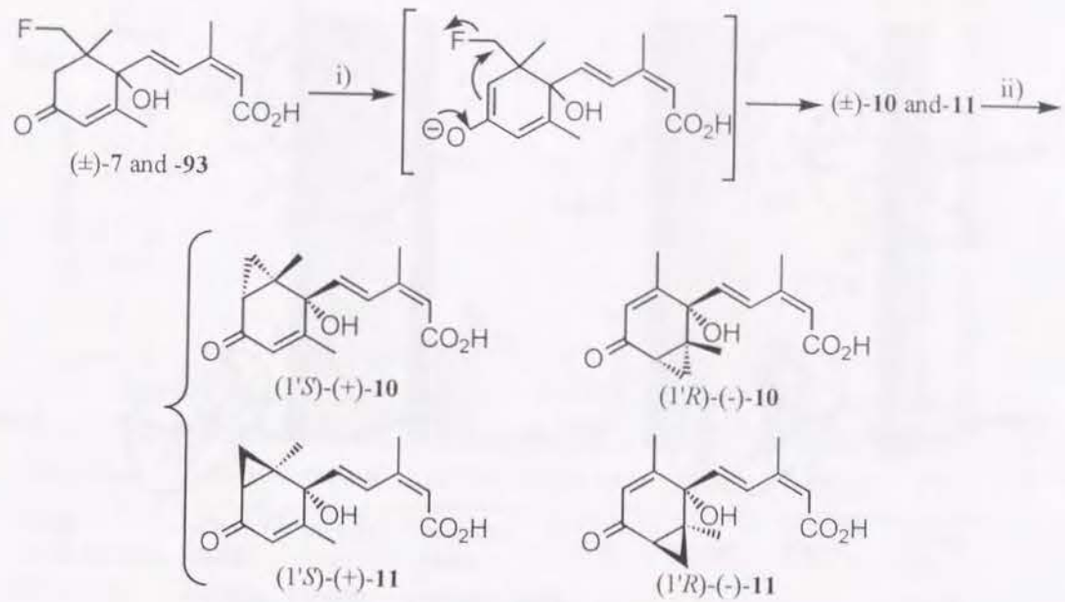


Fig. 24. Preparation of optically active **10** and **11**.
i) NaOH ii) HPLC with Chiralpak AD.

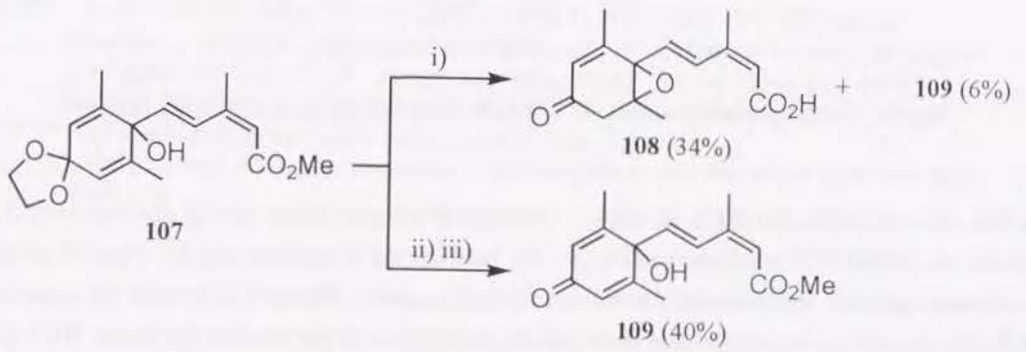
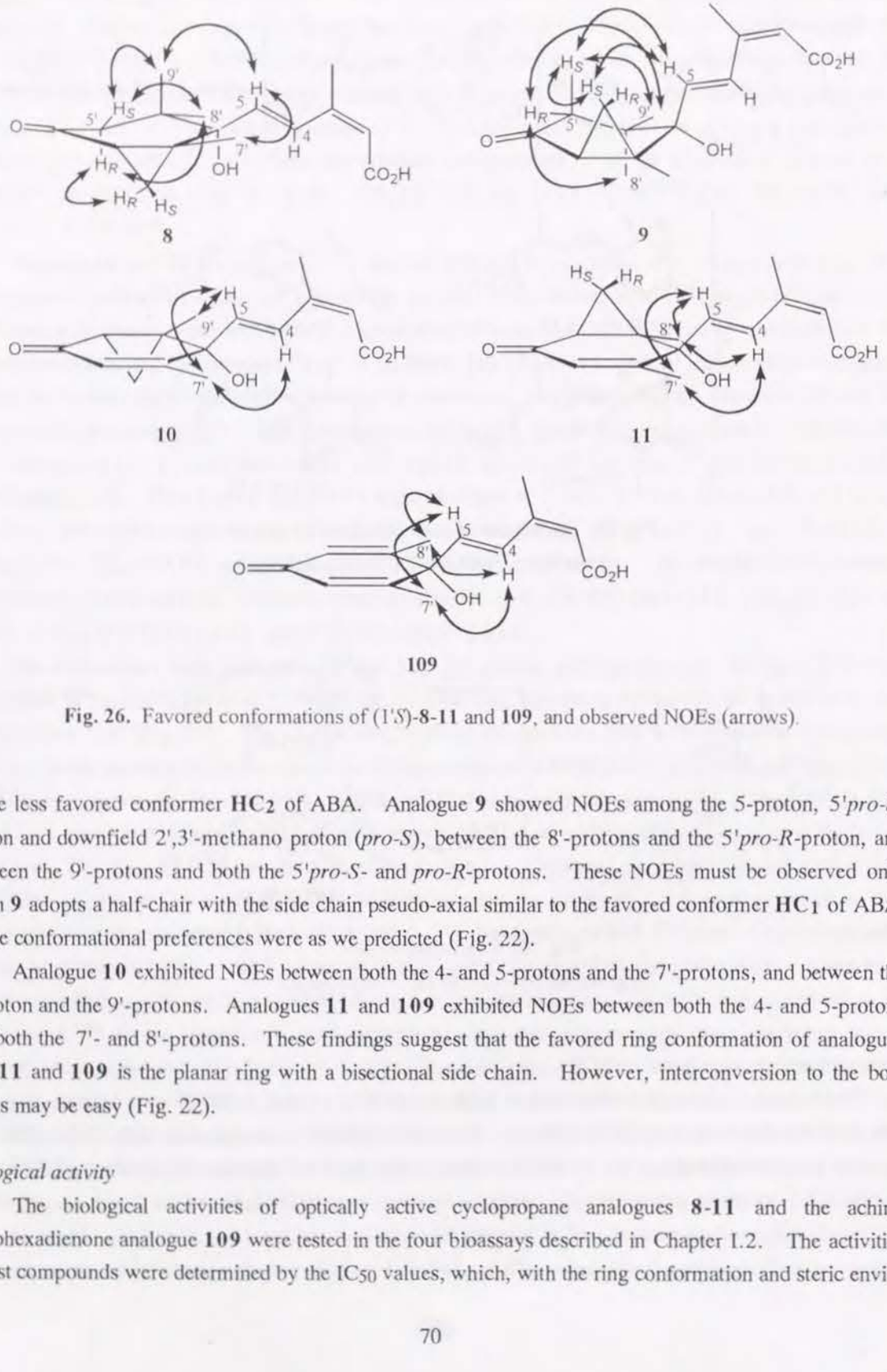


Fig. 25. Preparation of **109**.
i) a) NaOH b) HCl ii) HCl iii) esterase.

Conformational analysis based on NOEs

The favored conformations of analogues **8**-**11** and **109** in solution at 300 K were examined on the basis of NOEs observed in the NOE difference spectra and NOESY in methanol-d_4 (Fig. 26). The 5-proton of **8** exhibited NOEs to the 7'- and 9'-protons, and the 5'*pro-R*-proton showed an NOE to the downfield 2',3'-methano proton (*pro-R*). Additionally, there were NOEs between the 8'-protons and both the 5'*pro-S*- and *pro-R*-protons, as well as between the 9'-protons and the 5'*pro-S*-proton. These results suggest that the favored ring conformation of **8** is a half-chair with the pseudo-equatorial side chain, similar



8 **9** **10** **11** **109**

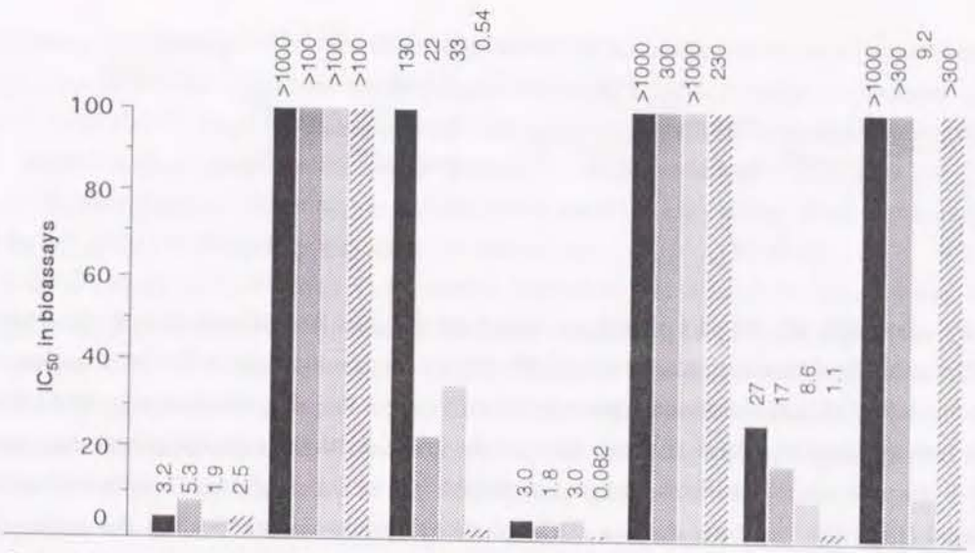
Fig. 26. Favored conformations of (1'*S*)-**8**-**11** and **109**, and observed NOEs (arrows).

to the less favored conformer **HC2** of ABA. Analogue **9** showed NOEs among the 5-proton, 5'*pro-S*-proton and downfield 2',3'-methano proton (*pro-S*), between the 8'-protons and the 5'*pro-R*-proton, and between the 9'-protons and both the 5'*pro-S*- and *pro-R*-protons. These NOEs must be observed only when **9** adopts a half-chair with the side chain pseudo-axial similar to the favored conformer **HC1** of ABA. These conformational preferences were as we predicted (Fig. 22).

Analogue **10** exhibited NOEs between both the 4- and 5-protons and the 7'-protons, and between the 5-proton and the 9'-protons. Analogues **11** and **109** exhibited NOEs between both the 4- and 5-protons and both the 7'- and 8'-protons. These findings suggest that the favored ring conformation of analogues **10**, **11** and **109** is the planar ring with a bisectional side chain. However, interconversion to the boat forms may be easy (Fig. 22).

Biological activity

The biological activities of optically active cyclopropane analogues **8**-**11** and the achiral cyclohexadienone analogue **109** were tested in the four bioassays described in Chapter I.2. The activities of test compounds were determined by the IC_{50} values, which, with the ring conformation and steric environment around the ring are summarized in Fig. 27, which also shows the activity of PA (**17**) (see Chapter I.2).

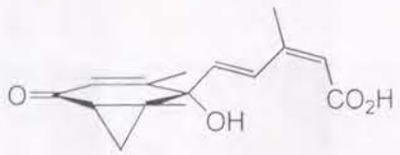


Compound	(1'*S*)-**1**	(1'*S*)-**8**	(1'*S*)-**9**	(1'*S*)-**10**	(1'*S*)-**11**	**109**	**17**
Ring conformation	pseudo-chair	pseudo-chair	pseudo-chair	planar	planar	planar	fixed chair
Side chain orientation	pseudo-axial	pseudo-equatorial	pseudo-axial	bisectional	bisectional	bisectional	fixed axial
axial C-2'β*	–	–	+	–	–	–	–
axial C-6'β*	–	+	–	–	+	–	–

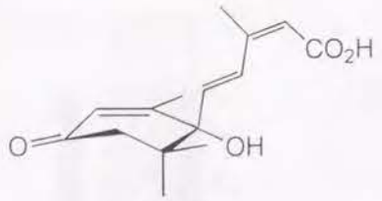
Fig.27. The IC_{50} of (1'*S*)-ABA (**1**), analogues (1'*S*)-**8**-**11**, **109** and (-)-PA (**17**) in four bioassays: stomatal opening of the epidermal strips of spiderwort (nM), ■; lettuce seed germination (μM), ▩; α-amylase induction by GA_3 in barley half-seeds (μM), ▨; elongation of the second 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.

In the stomata assay, analogues (1'*S*)-**8** and (1'*S*)-**11** were not active at all, whereas the activity of (1'*S*)-**10** was equivalent to that of (1'*S*)-ABA and those of (1'*S*)-**9** and **109** 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*)-**10**, which was similar to (1'*R*)-ABA, 1/2 to 1/10 of (1'*S*)-ABA (data not shown). PA was inactive except for the α-amylase assay.[188]

Key information for the ring conformational requirement can be drawn from a comparison of active (1'*S*)-**10**, inactive (1'*S*)-**11** and relatively active **109**. As mentioned above, analogues (1'*S*)-**10**, (1'*S*)-**11** and **109** possess similar, planar ring conformations, but different orientations of the 6'-substituents (Fig. 26); (1'*S*)-**10** always has the axial-like 6'α-substituent and the equatorial-like 6'β-substituent, (1'*S*)-**11** always has the equatorial-like 6'α-substituent and the axial-like 6'β-substituent, and **109** has only one equatorial-like 6'-methyl group. Thus the difference in the activity can be attributed to the steric environ-



(1'*R*)-**10**



(1'*R*)-ABA

ment at C-6'. Inactivity of (1'*S*)-**11** should be caused by the axial-like 6'β-substituent rather than the absence of the axial-like 6'α-substituent because **109** 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. 20). As the absence of the 6'-methyl groups is not fatal to the activity,[92,133] inactivation caused by the axial-like 6'β-methyl would depend on steric hindrance against binding to the receptor, suggesting that the 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[91]. 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 **HC2** with the side chain pseudo-equatorial as Milborrow speculated, because the half-chair **HC2** 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 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 **65** is active,[98] and (1'*R*)-ABA and (1'*R*)-**10** 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 **HC1** or **S1** and **S3** which can be adopted with a little increase of potential energy from the favored conformation.

The activity of the other (1'*S*)-analogues **8** and **9**, 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*)-**8** possesses the axial 6'β-methyl in its favored conformation. Although (1'*S*)-**8** possesses the axial 2'α-substituent, cyclopropane ring, it would be little responsible for the inactivity because (1'*R*)-**10**, which possesses the same axial substituent at the site corresponding to C-2'α based on the pseudo-symmetric hypothesis of the ABA molecule,[91,95] was relatively active. Analogue (1'*S*)-**9** that showed low activity possesses an axial-like cyclopropyl ring at the β-side of the ring. According to the pseudo-symmetric hypothesis, the inactive (1'*R*)-analogues, **8**, **9** and **11**, possess substituents corresponding to either the axial 6'β- or 2'β-substituent in (1'*S*)-ABA, while active (1'*R*)-**10** does possesses neither. The activity of the allenic analogue **96** and dihydro analogues **65** and **66** also can be explained by our supposed ring

conformational requirement. The side chain in inactive **96** is constrained to the equatorial and cannot change into the bisectional. Although the side chain in active **65** 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 **65** can adopt a conformation similar to the active conformation of ABA with a little increase of energy. On the other hand, **66** 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*)-**8** and (1'*S*)-**11** were as inactive as they were in the other assays, but (1'*S*)-**9**, (1'*S*)-**10** and **109** were more potent than (1'*S*)-ABA. Particularly, the 30-fold higher activity of (1'*S*)-**10** 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 (see Chapter I.2). These high activities seem to depend on slow metabolism; the weakened electrophilicity at C-2' for (1'*S*)-**9** and the fixed C-8' for (1'*S*)-**10** would resist the cyclization to inactive PA, and the lack of the axial 6'α-methyl for **109** would not afford a 8'-hydroxy-derivative to cyclize. It is of interest how (1'*S*)-**10** and **109** were 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 (see Chapter I.1). Analogue (1'*S*)-**8** seems to resist cyclization like (1'*S*)-**9**, but the activity of (1'*S*)-**8** 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*)-**8** has an axial substituent at C-6'β which is fatal to exhibiting the activity as described above.

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[95] and the role of the 1'-hydroxyl group as the hydrogen bonding donor (see Chapter II.2). 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 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.

(±)-4,4-*Ethylenedioxy*-2,3-*dihydro*-2,3-*methano*-2,6,6-*trimethylcyclohexan*-1-*one* (**98**)[185]

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 (**97**)[186] (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 **98** (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* (**99** and **100**)

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 **98** 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 **99** and **100** (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, $CDCl_3$): the signals of **99**: 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); the signals of **100**: 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 ($C_{18}H_{26}O_4$ requires 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* (**101** and **102**)

To a stirred solution of a mixture of **99** and **100** (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 **101** and **102** (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, $CDCl_3$): the signals of **101**: 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); the signals of **102**: 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 ($C_{18}H_{28}O_4$ requires 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* (**103** and **104**)

To a solution of a mixture of **101** and **102** (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 **103** and **104** (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 ^1H NMR spectrum. ^1H NMR (500 MHz, $CDCl_3$): the signals of **103**: 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); the signals of **104**: 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 ($C_{18}H_{26}O_4$ requires 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* (**105** and **106**)

To a solution of a mixture of **103** and **104** (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 **105** (0.21 g, 8.3 % yield) and **106** (1.76 g, 69.6% yield) as colorless oils. **105**: ^1H 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 ($C_{19}H_{28}O_5$ requires 336.1937). **106**: ^{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 ($C_{19}H_{28}O_5$ requires 336.1937).

(±)-*Methyl* 2'α,3'α-*dihydro*-2'α,3'α-*methanoabscisate* (**8a**) *and* (±)-*methyl* 2'β,3'β-*dihydro*-2'β,3'β-*methanoabscisate* (**9a**)

To a solution of **105** (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 **8a** (100 mg, 95.9% yield) as a white solid. In the similar manner to **105**, **106** (1.85 g, 5.5 mmol) gave **9a** (1.52 g, 94.5% yield) as a colorless oil. **8a**: ^{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 ν_{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 ($C_{17}H_{24}O_4$ requires 292.1675). **9a**: ^{1}H 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 ν_{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 ($C_{17}H_{24}O_4$ requires 292.1675).

(±)-2'α,3'α-*Dihydro*-2'α,3'α-*methano-ABA* (**8**) *and* (±)-2'β,3'β-*dihydro*-2'β,3'β-*methano-ABA* (**9**)

To a solution of **8a** (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 (±)-**8** (46.6 mg, 94.1% yield) as a white amorphous solid. In the similar manner to **8a**, **9a** (820 mg, 2.8 mmol) gave (±)-**9** (675 mg, 86.5% yield) as a colorless oil. (±)-**8**: ^{1}H 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 λ_{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 ($C_{16}H_{22}O_4$ requires 278.1518). (±)-**9**: ^{1}H 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 λ_{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 ($C_{16}H_{22}O_4$ requires 278.1518).

(±)-5'α,8'-*Cyclo-ABA* (**10**) *and* (±)-5'β,9'-*Cyclo-ABA* (**11**)

To a solution of (±)-**7** (see Chapter II.2) (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 (±)-**10** (47.3 mg) as a white amorphous solid. In the same manner as (±)-**7**, (±)-**93** (120 mg) gave (±)-**11** (110.2 mg) as a white amorphous solid. (±)-**10**: ^{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 λ_{max} (MeOH) nm (ε): 269 (17,100), 240 (15,400); IR of the methyl ester ν_{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 ($C_{16}H_{20}O_4$ requires 276.1362). (±)-**11**: ^{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 λ_{max} (MeOH) nm (ε): 246 (24,500); IR of the methyl ester ν_{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 ($C_{16}H_{20}O_4$ requires 276.1362).

(2Z,4E)-5-(2',6'-*Dimethyl*-1'-*hydroxy*-4'-*oxocyclohxa*-2',5'-*dienyl*)-3-*methylpent*-2,4-*dienoic acid* (**109**)

The methyl ester **109a** was synthesized from **107** according to the method reported by Lei *et al.*[149] To a solution of **109a** (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 **109** (26 mg, 69% yield, 40% yield from **107**) as a white amorphous powder. The spectral data of **109** agreed with those reported.

The conversion of **107** (40 mg) by the reported method gave 11 mg of **108** (34% yield) and 1.8 mg of **109** (6% yield). **108**: ^{1}H 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 λ_{max} (MeOH) nm (ε): 244 (21,300); FAB-MS (matrix, 3-nitrobenzyl alcohol) *m/z*: 249 $[M+H]^+$.

Stability of **109**

The aqueous solutions (3 x 10^{-4} M) of **109** 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 **109** and ABA were calculated to be 54 and 87%, respectively, by being compared with the peak-heights of the standard samples.

Optical resolution of **8**-**11**

Racemic **8** (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 (+)-**8** (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. (-)-**8**: $[\alpha]_D^{25}$ -21.4° (MeOH, *c* 0.673); CD λ_{ext} (MeOH) nm (Δε): 283.4 (-0.9), 255.4 (+3.3), 207.6 (-13.5). (+)-**8**: $[\alpha]_D^{25}$ +19.8° (MeOH, *c* 0.667); CD λ_{ext} (MeOH) nm (Δε): 284.6 (+1.1), 258.3 (-3.2), 205.6 (+14.5). Racemic **9** (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 (+)-**9** (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. (-)-**9**: $[\alpha]_D^{25}$ -68.0° (MeOH, *c* 0.447); CD λ_{ext} (MeOH) nm (Δε): 247.0 (-3.7), 212.7 (+3.3). (+)-**9**: $[\alpha]_D^{25}$ +67.3° (MeOH, *c* 0.440); CD λ_{ext} (MeOH) nm (Δε): 248.4 (+3.8), 214.0 (-2.5). Racemic **10** (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 (-)-**10** (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. (+)-**10**: $[\alpha]_D^{27}$ +527.5° ($CHCl_3$, *c* 0.333); CD λ_{ext} (MeOH) nm (Δε): 329.6 (+3.2), 270.7 (+32.3), 218.5 (-19.2). (-)-**10**: $[\alpha]_D^{27}$ -528.4° ($CHCl_3$, *c* 0.333); CD λ_{ext} (MeOH) nm (Δε): 329.2 (-3.5), 270.5 (-31.4), 217.2 (+19.1). Racemic **11** (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 (-)-**11** (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. (+)-**11**: $[\alpha]_D^{27}$ +200.2° ($CHCl_3$, *c* 0.213), +224.6° (MeOH, *c* 0.213); CD λ_{ext} (MeOH) nm (Δε): 313.2 (+5.4), 269.2 (-5.1), 239.4 (+16.5), 214.0 (-6.9). (-)-**11**: $[\alpha]_D^{27}$ -210.9° ($CHCl_3$, *c* 0.223), -207.3° (MeOH, *c* 0.223); CD λ_{ext} (MeOH) nm (Δε): 313.1 (-5.1), 269.0 (+4.8), 239.2 (-15.5), 213.1 (+7.3).

Preparation of optically active **10** *and* **11** *from optically active* **7** *and* **93**

To a solution of (+)-**7** (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 (-)-**10** under the same HPLC condition that racemic **10** was optically resolved. In the same manner as (+)-**7**, (-)-**7** gave (+)-**10**, (+)-**93** did (+)-**11** and (-)-**93** did (-)-**11**.

Bioassays

See Chapter I.2.

Summary

The plant hormone ABA has not been applied to agriculture because of drawbacks including its rapid metabolism to inactive PA via 8'-HOABA. This apparently indicated that a metabolically stable analogue can overcome the short half-life of ABA to enhance its activity. However, no analogue modified at C-8' that is hydroxylated at the first stage of the major metabolic pathway, has yet been synthesized. Two ways conferred resistance to metabolic inactivation; one was the blockage of hydroxylation to 8'-HOABA, and the other was cyclization to inactive PA.

The synthesized analogues 8',8'-difluoro and 8',8',8'-trifluoro-ABAs (**2** and **3**) were resistant to the 8'-hydroxylation (Chapter I.2). The metabolism of **2** and **3** was not investigated, but the fact that they showed the higher and longer-lasting activity in a long-term rice assay, suggested that the half-life was extended as a result of resistance to the 8'-hydroxylation. The 8'-trifluoro analogue **3** was over 30-fold more potent than ABA in inhibiting rice seedling elongation is the most potent analogue of ABA to date.

Analogues that can resist cyclization to PA were designed from two standpoints; a decrease in the nucleophilicity of the 8'-oxygen, and a decrease in the electrophilicity of the 2'-carbon. 8'-Methoxy-ABA (**4**) blocked the nucleophilic addition of the 8'-oxygen to the 2'-carbon, and its activity was lasted longer in the rice assay (Chapter I.3). 3'-Fluoro-ABA (**5**) blocked the electrophilic addition of the 2'-carbon to the 8'-oxygen due to the higher π-electron density at C-2' induced by the electron-donating effect of the 3'-fluorine (Chapter I.4). This analogue did not strengthen the activity, but the metabolite 3'-fluoro-8'-HOABA (**72**) was isolated as the methyl ester **72a** along with the methyl esters of 3'-fluoro-PAs (**73a** and **74a**) and 3'-fluoro-DPAs (**75a** and **76a**) when **5** was applied to bean shoots. At room temperature, **72a**-**74a** converted into an equilibrium mixture consisting of **72a**, **73a** and **74a** at a ratio of 7:6:1. This showed that the introduction of electron-donating group at C-3' in ABA causes resistance to cyclization, although with fluorine, the resistance was partial.

An alternative approach to development of highly active analogues of ABA is to design a high affinity analogue for the receptor based upon understanding of the structure-activity relationships. The author investigated the role of the 1'-hydroxyl group of ABA and the 8'-hydroxyl group of 8'-HOABA using 1'-deoxy-1'-fluoro-ABA (**6**) and 8'-fluoro-ABA (**7**) (Chapter II). The activity of **6** was 1/10 - 1/20 that of ABA and similar to that of 1'-deoxy-ABA (**79**), meaning that the 1'-hydroxyl group of ABA cannot be mimicked by the fluorine atom. This suggested that the 1'-hydroxyl group of ABA interacts with the receptor as the hydrogen-bonding donor. The activity of **7** was similar to that of ABA, suggesting that the 8'-hydroxyl group is not involved in activity.

The ring conformational requirement of ABA for activity was probed using the cyclopropane analogues **8**-**11** (Chapter III). Analogues **8**, **9** and **11** which possess the axial substituent at C-6' or C-2' on the β-side of the ring showed no or very low activity, whereas **10**, which does not possess these substituents, exhibited the activity comparable to that of ABA. This suggested that the active conformation of ABA is close to the favored half-chair with a pseudo-axial side chain and an equatorial C-9' rather than the less favored half-chair with the former pseudo-equatorial and the latter axial. These findings will be useful in developing highly potent analogues and photoaffinity probes with which to search for the ABA receptor. Also, analogue **10** showed high activity in the rice assay, equal to that of **3**.

The author developed the first highly potent, long-lasting analogues of ABA. These analogues will be useful not only as plant growth regulators but also as probes with which to investigate the mechanism of ABA action.

Acknowledgments

This study was carried out in the Laboratory of Analysis of Agricultural Products, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, from 1991 to 1996.

The author wishes to thank Dr. Hajime Ohigashi, Professor of Kyoto University, for his encouragement and suggestions.

The author greatly appreciates Dr. Koichi Koshimizu, emeritus professor of Kyoto University, for his guidance.

The author is grateful to Dr. Nobuhiro Hirai for his guidance, encouragement and practical advice which have been indispensable to the continuation of this study. The author also thanks Dr. Kazuhiro Irie of the staff of the Laboratory of Analysis of Agricultural Products for his suggestions and discussion.

The author thanks Dr. Masahiko Okamoto of Sumitomo Chemical Co., Ltd. for screening the chiral columns, measuring the NOESY and ^{13}C NMR spectra, as well as for his advice, Professor Junichi Ueda of University of Osaka Prefecture for supplying the lettuce and barley seeds, the spiderwort and for his encouragement, Professor Susumu Yazawa of Faculty of Agriculture at Kyoto University for the advice about the cultivation of bean plants, Dr. Hiroshi Okumoto of Faculty of Agriculture at Kyoto University for supplying the rice seeds, Mr. Ryuichi Imamura of Faculty of Science at Kyoto University for ^{1}H NMR measurements, and Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan for the gift of the (+)-ABA. The author thanks Mr. Sei-ichi Nakano for his collaboration. Acknowledgments are also made to all of the members of the Laboratory of Analysis of Agricultural Products. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

Finally, the author owes special thanks to his parents and family for their warm encouragement and financial support.

References and Notes

1. Addicott, F.T. and Carns, H.R. (1983) History and introduction. In *Abscisic Acid* (Addicott, F.T., ed.). Praeger, New York, pp. 1-21
2. Zeevaart, J.A.D. and Creelman, R.A. (1988) Metabolism and physiology of abscisic acid. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 439-473.
3. Davies, W.J. and Jones, H.G., eds (1991) *Abscisic Acid*. BIOS Scientific Publishers Ltd., Oxford.
4. Zeevaart, J.A.D., Rock, C.D., Fantauzzo, F., Heath, T.G. and Gage, D.A. (1991) Metabolism of ABA and its physiological implications. In *Abscisic Acid* (Davies, W.J. and Jones, H.G., eds). BIOS Scientific Publishers Ltd., Oxford, pp. 39-52.
5. Parry, A.D. and Horgan, R. (1991) Carotenoids and abscisic acid (ABA) biosynthesis in higher plants. *Physiol. Plant.* **82**, 320-326.
6. Walton, D.C. and Li, Y. (1995) Abscisic acid biosynthesis and metabolism. In *Plant Hormones* (Davies, P.J., ed.). Kluwer Academic Publishers, Dordrecht, pp. 140-157.
7. McAinsh, M.R., Brownlee, C., Sarsag, M., Webb, A.A.R. and Hetherington, A.M. (1991) Involvement of second messengers in the action of ABA. In *Abscisic Acid* (Davies, W.J. and Jones, H.G., eds). BIOS Scientific Publishers Ltd., Oxford, pp. 137-152.
8. Bowler, C. and Chua, N.-H. (1994) Emerging themes of plant signal transduction. *Plant Cell* **6**, 1529-1541.
9. Thomas, T.L., Vivekananda, J. and Bogue, M.A. (1991) ABA regulation of gene expression in embryos and mature plants. In *Abscisic Acid* (Davies, W.J. and Jones, H.G., eds). BIOS Scientific Publishers Ltd., Oxford, pp. 125-136.
10. Chandler, P.M. and Robertson, M. (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 113-141.
11. Bray, E.A. (1991) Regulation of gene expression by endogenous ABA during drought stress. In *Abscisic Acid* (Davies, W.J. and Jones, H.G., eds). BIOS Scientific Publishers Ltd., Oxford, pp. 81-98.
12. Close, T.J., Kortt, A.A. and Chandler, P.M. (1989) A cDNA-based comparison of dehydration-induced proteins (Dehydrins) in barley and corn. *Plant Mol. Biol.* **13**, 95-108.
13. Sánchez-Serrano, J.J., Amati, S., Ebneth, M., Hildmann, T., Mertens, R., Peña-Cortés, H. Prat, S. and Willmitzer, L. (1991) The involvement of ABA in wound responses of plants. In *Abscisic Acid* (Davies, W.J. and Jones, H.G., eds). BIOS Scientific Publishers Ltd., Oxford, pp. 201-216.
14. Leung, J., Bouvier-Durand, M., Morris, P.-C., Guerrier, D., Chefdor, F. and Giraudat, J. (1994) *Arabidopsis* ABA response gene *ABI1*: features of a calcium-modulated protein phosphatase. *Science* **264**, 1448-1452.
15. Meyer, K., Leube, M.P. and Grill, E. (1994) A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. *Science* **264**, 1452-1455.

16. Hartung, W. and Slovik, S. (1991) Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: stomatal regulation by abscisic acid in leaves. *New Phytol.* **119**, 361-382.
17. Loveys, B.R. (1991) How useful is a knowledge of ABA physiology for crop improvement? In *Abscisic Acid* (Davies, W.J. and Jones, H.G., eds). BIOS Scientific Publishers Ltd., Oxford, 245-260.
18. Willows, R.D. and Milborrow, B.V. (1993) Synthesis and properties of C-1-azido-ABA. *Phytochemistry* **32**, 869-873.
19. Plancher, B. (1979) Anmerkungen zur UV-Isomerisation der Abscisinsäure. *Gartenbauwissenschaft* **44**, 184-191.
20. Brabham, D.E. and Biggs, R.H. (1981) *Cis-trans* photoisomerization of abscisic acid. *Photochemistry and Photobiology* **34**, 33-37.
21. Cornelussen, M.H.M., Karssen, C.M. and van Loon, L.C. (1995) UV-induced cross-linking of abscisic acid to binding proteins. *Phytochemistry* **39**, 959-968.
22. Chen, S.C. and MacTaggart, J.M. (1986) Abscisic acid analogs with a geometrically rigid conjugated acid side-chain. *Agric. Biol. Chem.* **50**, 1097-1100.
23. Ohkuma, K. (1966) Synthesis of some analogs of abscisin II. *Agric. Biol. Chem.* **5**, 434-437.
24. Lehmann, H. and Schütte, H.R. (1980) Purification and characterization of an abscisic acid glucosylating enzyme from cell suspension cultures of *Macleaya microcarpa*. *Z. Pflanzenphysiol.* **96**, 277-280.
25. Lehmann, H. and Glund, K. (1986) Abscisic acid metabolism—vacuolar/extravacuolar distribution of metabolites. *Planta* **168**, 559-562.
26. Kriedmann, P.E., Loveys, B.R., Fuller, G.L. and Leopold, A.C. (1972) Abscisic acid and stomatal regulation. *Plant Physiol.* **49**, 842-847.
27. Koshimizu, K., Fukui, H., Mitsui, T. and Ogawa, Y. (1966) Identify of lupin inhibitor with abscisin II and its biological activity on growth of rice seedlings. *Agric. Biol. Chem.* **32**, 789-791.
28. Walton, D.C. and Sondheimer, E. (1972) Activity and metabolism of ^{14}C-(±)-abscisic acid derivatives. *Plant Physiol.* **49**, 290-292.
29. Jones, R.J. and Mansfield, T.A. (1972) Effects of abscisic acid and its esters on stomatal aperture and the transpiration ratio. *Physiol. Plant.* **26**, 321-327.
30. Loveys, B.R. and Milborrow, B.V. (1981) Isolation and characterization of 1'-*O*-abscisic acid-β-D-glucopyranoside from vegetative tomato tissue. *Aust. J. Plant Physiol.* **8**, 571-589.
31. Beale, M.H., Chen, A., Harrison, P.A. and Willis, C.L. (1993) Facile aromatisation of abscisic acid. *J. Chem. Soc. Perkin Trans. 1*, 3061-3064.
32. Davis, L.A., Heinz, D.E. and Addicott, F.T. (1968) Gas-liquid chromatography of trimethylsilyl derivatives of abscisic acid and other plant hormones. *Plant Physiol.* **43**, 1389-1394.
33. Most, B.H., Gaskin, P. and MacMillan, J. (1970) The occurrence of abscisic acid in inhibitors B_1 and C from immature fruit of *Ceratonia siliqua* L. (carob) and in commercial carob syrup. *Planta* **92**, 41-49.

34. Terem, B. and Utley, H.P. (1979) Electro-organic reactions—14. The intramolecular cathodic cyclization of abscisic acid methyl ester. *Electrochimica Acta* **24**, 1081-1084.
35. Loveys, B.R. and Milborrow, B.V. (1984) Metabolism of abscisic acid. In *The biosynthesis and metabolism of plant hormones* (Crozier, A. and Hillman, J.R. eds). Cambridge University Press, New York, pp. 71-104.
36. Milborrow, B.V. (1983) Pathways to and from abscisic acid. In *Abscisic Acid* (Addicott, F.T., ed.). Praeger, New York, pp. 79-111.
37. Hirai, N., Fukui, H. and Koshimizu, K. (1978) A novel abscisic acid metabolite from seeds of *Robinia pseudacacia*. *Phytochemistry* **17**, 1625-1627.
38. Gillard, D.F. and Walton, D.C. (1976) Abscisic acid metabolism by a cell-free preparation from *Echinocystis lobata* Liquid Endosperm. *Plant Physiol.* **58**, 790-795.
39. Bolwell, G.P., Bozak, K. and Zimmerlin, A. (1994) Plant cytochrome P450. *Phytochemistry* **37**, 1491-1506, and references cited therein.
40. Creelman, R.A. and Zeevaart, J.A.D. (1984) Incorporation of oxygen into abscisic acid and phaseic acid from molecular oxygen. *Plant Physiol.* **75**, 166-169.
41. Creelman, R.A., Gage, D.A., Stults, G. and Zeevaart, J.A.D. (1987) Abscisic acid biosynthesis in leaves and roots of *Xanthium strumarium*. *Plant Physiol.* **85**, 726-732.
42. Uknes, S.J. and Ho, T.-H.D. (1984) Mode of action of abscisic acid in barley aleurone layers. Abscisic acid induces its own conversion to phaseic acid. *Plant Physiol.* **75**, 1126-1132.
43. Lehmann, H., Preiss, A. and Schmidt, J. (1983) A novel abscisic acid metabolite from cell suspension cultures of *Nigella damascena*. *Phytochemistry* **22**, 1277-1278.
44. Railton, I.D. and Cowan, A. K. (1985) 2'-Hydroxymethyl abscisic acid: a major catabolite of (±)-abscisic acid in leaves of *Hordeum vulgare* L. *Plant Sci.* **42**, 169-172.
45. Cowan, A.K. and Railton, I.D. (1987) The catabolism of (±)-abscisic acid by excised leaves of *Hordeum vulgare* L. cv Dyan and its modification by chemical and environmental factors. *Plant Physiol.* **84**, 157-163.
46. Boyer, G.L. and Zeevaart, J.A.D. (1986) 7'-Hydroxy (-)-*R*-abscisic acid: a metabolite of feeding (-)-*R*-abscisic acid to *Xanthium strumarium*. *Phytochemistry* **25**, 1103-1105.
47. Balsevich, J.J., Abrams, S.R., Lamb, N. and König, W.A. (1994) Identification of unnatural phaseic acid as a metabolite derived from exogenously added (-)-abscisic acid in a maize cell suspension culture. *Phytochemistry* **36**, 647-650.
48. Hampson, C.R., Reaney, M.J.T., Abrams, G.D., Abrams, S.R. and Gusta, L.V. (1992) Metabolism of (+)-abscisic acid to (+)-7'-hydroxyabscisic acid by bromegrass cell cultures. *Phytochemistry* **31**, 2645-2648.
49. Dashek, W.V., Singh, B.N. and Walton, D.C. (1979) Abscisic acid localization and metabolism in barley aleurone layers. *Plant Physiol.* **64**, 43-48.
50. Okamoto, M. and Nakazawa, H. (1993) Stereoselectivity of abscisic acid-oxygenase in avocado fruits. *Biosci. Biotech. Biochem.* **57**, 1768-1769.
51. Lehmann, H. and Schwenen, L. (1988) Nigellic acid—An endogenous abscisic acid metabolite from *Vicia faba* leaves. *Phytochemistry* **27**, 677-678.

52. Loveys, B. and Milborrow, B.V. (1992) Hydroxylation of methyl abscisate and formation of three β-D-glucosides. *Phytochemistry* **31**, 67-72.

53. Balsevich, J.J., Cutler, A.J., Lamb, N., Friesen, L.J., Kurz, E.U., Perras, M.R. and Abrams, S.R. (1994) Responce of cultured maize cells to (+)-abscisic acid, (-)-abscisic acid, and their metabolites. *Plant Physiol.* **106**, 135-142.

54. Milborrow, B.V. (1968) Identification and measurement of (+)-abscisic acid in plant. In *Biochemistry and Physiology of Plant Growth Substances* (Wightman, F. and Setterfield, G. eds). Runge Press, Ottawa, pp. 1531-1545.

55. Adesomoju, A.A., Okogun, J.I., Ekong, D.E.U. and Gaskin, P. (1980) GC-MS identification of abscisic acid and abscisic acid metabolites in seed of *Vigna unguiculata*. *Phytochemistry* **19**, 223-225.

56. Zou, J., Abrams, G.D., Barton , D.L., Taylor, D.C., Keith Pomeroy, M. and Abrams, S.R. (1995) Induction of lipid and oleosin biosynthesis by (+)-abscisic acid and its metabolites in microspore-derived embryos of *Brassica napus* L. cv Reston: biological responses in the presence of 8'-hydoxyabscisic acid. *Plant Physiol.* **108**, 563-571.

57. Milborrow, B.V., Carrington, N.J. and Vanghan, G.T. (1988) The cyclization of 8'-hydroxy abscisic acid to phascic acid *in vivo*. *Phytochemistry* **27**, 757-759.

58. Häuser, C., Kwiatkowski, J., Rademacher, W. and Grossmann, K. (1990) Regulation of endogenous abscisic acid levels and transpiration in oilseed rape by plant growth retardants. *J. Plant Physiol.* **137**, 201-207.

59. Belefant, H. and Fong, F. (1991) Abscisic acid biosynthesis in *Zea mays* embryos: influence of tetcyclacis and regulation by osmotic potential. *Plant Sci.* **78**, 19-25.

60. Black, S.D. (1987) P-450 cytochromes: structure and function. In *Advances in enzymology and related areas of molecular biology* (Meister, A. ed.). John Wiley & Sons, New York, pp. 35-87.

61. Weast, R.C. ed. (1988) *Handbook of chemistry and physics*. CRC Press, Boca Raton.

62. Tanaka, Y., DeLuca, H.F., Kobayashi, Y. and Ikekawa, N. (1984) 26,26,26,27,27,27-Hexafluoro-1,25-dihydroxyvitamin D_3: a highly potent, long-lasting analog of 1,25-dihydroxyvitamin D_3. *Arch. Biochem. Biophys.* **229**, 348-354.

63. Okamoto, S., Tanaka, Y., DeLuca, H.F., Kobayashi, Y. and Ikekawa, N. (1983) Biological activity of 24,24-difluoro-1,25-dihydroxyvitamin D_3. *Am. J. Physiol.* **244**, E159.

64. Asai, T., Morizawa, Y., Shimada, T., Nakayama, T., Urushihara, M., Matsumura, Y. and Yasuda, A. (1995) Synthesis of new stable fluoroprostacyclin analogs with potent anti-anginal activity. *Tetrahedron Lett.* **36**, 273-276.

65. Welch, J.T. (1987) Advances in the preparation on biologically active organofluorine compounds. *Tetrahedron* **43**, 3123-3197.

66. McClinton, M.A. and McClinton, D.A. (1992) Trifluoromethylations and related reactions in organic chemistry. *Tetrahedron* **48**, 6555-6666.

67. Resnati, G. (1993) Synthesis of chiral and bioactive fluoroorganic compounds. *Tetrahedron* **49**, 9385-9445.

68. Rozen, S. and Filler, R. (1985) α-Fluorocarbonyl compounds and related chemistry. *Tetrahedron* **41**, 1111-1153.

69. Gerstenberger, M.R.C. and Haas, A. (1981) Methods of fluorination in organic chemistry. *Angew. Chem. Int. Ed. Engl.* **20**, 647-667.

70. Purrington, S.T., Kagen, B.S. and Patrick, T.B. (1986) The application of elemental fluorine in organic synthesis. *Chem. Rev.* **86**, 997-1018.

71. Wilkinson, J.A. (1992) Recent advances in the selective formation of the C-F bond. *Chem. Rev.* **92**, 505-519.

72. Rose, P.A., Abrams, S.R. and Gusta, L.V. (1992) Synthesis, resolution and biological activity of 7',7'-difluoroabscisic acid. *Phytochemistry* **31**, 1105-1110.

73. Takahashi, S., Oritani, T. and Yamashita, K. (1986) Total synthesis of (±)-methyl phascates. *Agric. Biol. Chem.* **50**, 1589-1595.

74. Grieco, P.A. and Hiroi, K. (1972) α-Hydroxymethylation of γ- and δ-lactones: a new synthesis of α-methylene-γ-butyrolactones. *J. Chem. Soc. Chem. Comm.*, 1317-1318.

75. Hanzawa, Y., Suzuki, M., Kobayashi, Y. and Taguchi, T. (1991) Construction of trifluoromethylated quarternary carbons via Diels-Alder reaction of 2-(trifluoromethyl)propenoic acid derivatives: application to the synthesis of 16,16,16-trifluororetinal. *J. Org. Chem.* **56**, 1718-1725.

76. Milborrow, B.V. (1984) The conformation of abscisic acid by n.m.r. and a revision of the proposed mechanism for cyclization during its biosynthesis. *Biochem. J.* **220**, 325-332.

77. Koreeda, M., Weiss, G. and Nakanishi, K. (1973) Absolute configuration of natural (+)-abscisic acid. *J. Am. Chem. Soc.* **95**, 239-240.

78. McWha, J.A., Philipson, J.J., Hillman. J.R. and Wilkins, M.B. (1973) Molecular requirements for abscisic acid activity in two bioassay systems. *Planta* **109**, 327-336.

79. Koshimizu, K., Fukui, H., Mitsui, T. and Ogawa, Y. (1966) Identity of lupin inhibitor with abscisin II and its biological activity on growth of rice seedlings. *Agric. Biol. Chem.* **30**, 941-943.

80. Coombe, B.G., Cohen, D. and Paleg, L.G. (1967) Barley endosperm bioassay for gibberellins. I. Parameters of the response system. *Plant Physiol.* **42**, 105-112.

81. Tucker, D.J. and Mansfield, T.A. (1971) A simple bioassay for detecting "antitranspirant" activity of naturally occurring compounds such as abscisic acid. *Planta* **98**, 157-163.

82. Orlandini, M., Barthe, Ph. and Bulard, C. (1984) Metabolism of (±) 2-[^{14}C]-abscisic acid in *Lectuca sativa* achenes. *Physiol. Plant.* **62**, 553-560.

83. Lin, L.-S. and Ho, T.-H.D. (1986) Mode of action of abscisic acid in barley aleurone layers. Induction of new proteins by abscisic acid. *Plant Physiol.* **82**, 289-297.

84. Walton, D.C. (1983) Structure-activity relationships of abscisic acid analogs and metabolites. In *Abscisic Acid* (Addicott, F.T., ed.). Praeger, New York, pp. 113-146, and references cited therein.

85. Marcotte, P.A. and Robinson, C.H. (1982) Design of mechanism-based inactivators of human placental aromatase. *Cancer Res.* **42**, 3322s-3326s.

86. Marumo, S., Lee, S.-K. and Kamuro, Y. (1990) Tennengata kogakukassei abusijinsan no seisan to kassei. *Shokubutsu Saibo Kogaku* (in Japanese) **2**, 503-513.

87. Sondheimer, E., Galson, E.C., Chang, Y.P. and Walton, D.C. (1971) Asymmetry, its importance to the action and metabolism of abscisic acid. *Science* **174**, 829-831.

88. Mertens, R., Stüning, M. and Weiler, E.W. (1982) Metabolism of tritiated enantiomers of abscisic acid prepared by immunoaffinity chromatography. *Naturwissenschaften* **69**, 595-597.

89. Abrams, S.R., Reaney, M.J.T., Abrams, G.D., Mazurek, T., Shaw, A.C. and Gusta, L.V. (1989) Ratio of (*S*)- to (*R*)-abscisic acid from plant cell cultures supplied with racemic ABA. *Phytochemistry* **28**, 2885-2889.

90. Dunstan, D.I., Bock, C.A., Abrams, G.D. and Abarms, S.R. (1992) Metabolism of (+)- and (-)-abscisic acid by somatic embryo suspension cultures of white spruce. *Phytochemistry* **31**, 1451-1454.

91. Milborrow, B.V. (1986) The shapes of abscisic acid and the active site. In *Plant Growth Substances 1985* (Bopp, M. ed.). Springer-Verlag, Berlin, pp. 108-119.

92. Nanzyo, M., Oritani, T. and Yamashita, K. (1977) Synthesis and physiological activity of monodemethyl abscisic acids and methyl 5-(1',6'-epoxy-2',2'-dimethylcyclohexyl)-3-methyl-(2*Z*,4*E*)-2,4-pentadienoate. *Agric. Biol. Chem.* **41**, 1711-1720.

93. Walker-Simmons, M.K., Rose, P.A., Shaw, A.C. and Abrams, S.R. (1994) The 7'-methyl group of abscisic acid is critical for biological activity in wheat embryo germination. *Plant Physiol.* **106**, 1279-1284.

94. Somogyi, M. (1952) Notes on sugar determination. *J. Biol. Chem.* **195**, 19-23.

95. Nakano,S., Todoroki, Y., Hirai, N. and Ohigashi, H. (1995) Synthesis and biological activity of 7'-, 8'-, and 9'-alkyl analogues of abscisic acid. *Biosci. Biotech. Biochem.* **59**, 1699-1706.

96. Oritani, T. and Yamashita, K. (1982) Synthesis and biological activity of (±)-2',3'-dihydro-abscisic acid. *Agric. Biol. Chem.* **46**, 817-818.

97. Lamb, N. and Abrams, S.R. (1990) Synthesis of optically active cyclohexanone analogs of the plant hormone abscisic acid. *Can. J. Chem.* **68**, 1151-1162.

98. Churchill, G.C., Ewan, B., Reaney, M.J.T., Abrams, S.R. and Gusta, L.V. (1992) Structure-activity relationships of abscisic acid analogs based on the induction of freezing tolerance in bromegrass (*Bromus inermis* Leyss) cell cultures. *Plant Physiol.* **100**, 2024-2029.

99. Lamb, N., Shaw, A.C., Abrams, S.R., Reaney, M.J.T., Ewan, B., Robertson, A.J. and Gusta, L.V. (1993) Oxidation of the 8'-position of a biologically active abscisic acid analogue. *Phytochemistry* **34**, 905-917.

100. Walton, D.C. and Sondheimer, E. (1972) Activity and metabolism of ^{14}C-(±)-abscisic acid derivatives. *Plant Physiol.* **49**, 290-292.

101. Oritani, T. and Yamashita, K. (1974) Synthsis and biological activities of analogs abscisic acid. *Agric. Biol. Chem.* **38**, 801-808.

102. House, H.O. and Ro, R.S. (1958) The stereochemistry of base-catalyzed epoxidation. *J. Am. Chem. Soc.* **80**, 2428-2433.

103. Miyashita, M., Suzuki, T. and Yoshikoshi, A. (1987) Fluoride-promoted epoxidation of α,β-unsaturated compounds. *Chem. Lett.* **46**, 4247-4254.

104. Suga, H., Hamatani, T. and Schlosser, M. (1990) Regioselective formation of fluorohydrins and their stereoselective conversion to fluoroolefins. *Tetrahedron* **46**, 4247-4254.

105. ^{13}C NMR (125 MHz) data for (±)-ABA in acetone-d_6: δ 19.0 (C-7'), 21.0 (C-6), 23.3 (C-8'), 24.5 (C-9'), 42.0 (C-6'), 50.2 (C-5'), 79.8 (C-1'), 118.4 (C-2), 127.1 (C-3'), 128.4 (C-4), 138.1 (C-5), 150.8 (C-3), 163.0 (C-2'), 167.0 (C-1), 197.1 (C-4').

106. Breitmaier, E. and Voelter, W. (1987) ^{13}C NMR spectral parameters and structural properties. In *Carbon-13 NMR Spectroscopy.*, VHC Verlagsgesellschaft, Weinheim, pp.107-182.

107. Seeley, S.D. and Powell, L.E. (1970) Electron capture-gas chromatography for sensitive assay of abscisic acid. *Anal. Biochem.* **35**, 530-533.

108. Milborrow, B.V. (1975) The absolute configuration of phaseic and dihydrophaseic acids. *Phytochemistry* **14**, 1045-1053.

109. Bowden, K., Heilbron, I.M., Jones, E.R.H. and Weedon, B.C.L. (1946) Researches on acetylenic compounds. Part. I. The preparation of acetylenic ketones by oxidation of acetylenic carbinols and glycols. *J. Chem. Soc.*, 39-45.

110. Corey, E.J. and Sneen, R.A. (1956) Stereoelectronic control in enolization-ketonization reactions. *J. Am. Chem. Soc.* **78**, 6269-6278.

111. Reichardt, C. (1979) Solvent effects on the position of homogeneous chemical equilibria. In *Solvent Effects in Organic Chemistry.*, Verlag Chemie, Weinheim, 53-77.

112. Zeevaart, J.A.D. and Milborrow, B.V. (1976) Metabolism of abscisic acid and the occurrence of *epi*-dihydrophaseic acid in *Phaseolus vulgaris*. *Phytochemistry* **15**, 493-500.

113. Weselake, R.J. and Jain, J.C. (1992) Strategies in the purification of plant proteins. *Physiol. Plant.* **84**, 301-309.

114. Hornberg, C. and Weiler, E.W. (1984) High-affinity binding sites for abscisic acid on the plasmalemma of *Vicia faba* guard cells. *Nature* **310**, 321-324.

115. Allan, A.C., Fricker, M.D., Ward, J.L., Beale, M.H. and Trewavas, A.J. (1994) Two transduction pathways mediate rapid effects of abscisic acid *Commelina* guard cells. *Plant Cell* **6**, 1319-1328.

116. Schwartz, A., Wu, W.-H., Tucker, E.B. and Assmann, S.M. (1994) Inhibition of inward K^+ channels and stomatal response by abscisic acid: an intracellular locus of phytohormone action. *Proc. Natl. Acad. Sci. USA* **91**, 4019-4023.

117. Anderson, B.E., Ward, J.M. and Schroeder, J.I. (1994) Evidence for an extracellular reception site for abscisic acid in *Commelina* guard cells. *Plant Physiol.* **104**, 1177-1183.

118. Assmann, S.M. (1994) Ins and outs of guard cell ABA receptors. *Plant cell* **6**, 1187-1190.

119. Gilroy, S. and Jones, R.L. (1994) Perception of gibberellin and abscisic acid at the external face of the plasma membrane of barley (*Hordeum vulgare* L.) aleurone protoplasts. *Plant Physiol.* **104**, 1185-1192.

120. Walker-Simmons, M.K., Reaney, M.J.T., Quarrie, S.A., Perata, P., Vernieri, P. and Abrams, S.R. (1991) Monoclonal antibody recognition of abscisic acid analogs. *Plant Physiol.* **95**, 46-51.

121. Hite, D.R.C., Outlaw, Jr., W.H. and Seavy, M.A. (1994) Substitution of hydrazones for the 4' carbonyl on abscisic acid rendered it ineffective in a rapid stomatal-opening-inhibition bioassay. *Physiol. Plant.* **92**, 79-84.

122. Astle, M.C. and Rubery, P.H. (1980) A study of abscisic acid uptake by apical and proximal root segments of *Phaseolus coccineus* L. *Planta* **150**, 312-320.

123. Astle, M.C. and Rubery, P.H. (1983) Carriers for abscisic acid and indole-3-acetic acid in primary roots: their regional localisation and thermodynamic driving forces. *Planta* **157**, 53-63.

124. Milborrow, B.V. and Rubery, P.H. (1985) The specificity of the carrier-mediated uptake of ABA by root segments of *Phaseolus coccineus* L. *J. Exp. Bot.* **36**, 807-822.

125. Daeter, W. and Hartung, W. (1993) The parmeability of the epidermal cell plasma membrane of barley leaves to abscisic acid. *Planta* **191**, 41-47.

126. Astle, M.C. and Rubery, P.H. (1985) Uptake of abscisic acid by suspension-cultured *Phaseolus coccineus* L. cells: evidence for carrier participation. *J. Exp. Bot.* **36**, 469-484.

127. Astle, M.C. and Robery, P.H. (1987) Carrier-mediated ABA uptake by suspension-cultured *Phaseolus coccineus* L. cells: stereospecificity and inhibition by ionones and ABA esters. *J. Exp. Bot.* **38**, 150-163.

128. Bianco-Colomas, J., Barthe, P., Orlandini, M. and Le Page-Degivry, M.-Th. (1991) Carrier-mediated uptake of abscisic acid by suspension-cultured *Amaranthus tricolor* cells. *Plant Physiol.* **95**, 990-996.

129. Windsor, M.L., Milborrow, B.V. and McFarlane, I.J. (1992) The uptake of (+)-*S*- and (-)-*R*-abscisic acid by suspension culture cells of hopbush (*Dodonaea viscosa*). *Plant Physiol.* **100**, 54-62.

130. Windsor, M.L., Milborrow, B.V. and Abrams, S.R. (1994) Stereochemical requirements of saturable uptake carrier for abscisic acid in carrot suspension culture cells. *J. Exp. Bot.* **45**, 227-233.

131. Perras, M.R., Abrams, S.R. and Balsevich, J.J. (1994) Characterization of an abscisic acid carrier in suspension-cultured barley cells. *J. Exp. Bot.* **45**, 1565-1573.

132. Oritani, T. and Yamashita, K. (1970) Studies on abscisic acid Part I. Synthesis of keto-ionylideneacetic acids. *Agric. Biol. Chem.* **34**, 108-114.

133. Nagano, E., Oritani, T. and Yamashita, K. (1980) Synthesis and physiological activity of methyl 6',6'-didemethyl abscisate and new chiral analogs. *Agric. Biol. Chem.* **44**, 2095-2098.

134. Yamashita, K., Watanabe, T., Watanabe, M. and Oritani, T. (1982) Synthesis and biological activity of methyl 3-demethyl-abscisate and its related analogs. *Agric. Biol. Chem.* **46**, 3069-3037.

135. Walker-Simmons, M.K., Anderberg, R.J., Rose, P.A. and Abrams, S.R. (1992) Optically pure abscisic acid analogs—Tools for relating germination inhibition and gene expression in wheat embryos. *Plant Physiol.* **99**, 501-507.

136. Wilen, R.W., Hays, D.B., Mandel, R.M., Abrams, S.R. and Moloney, M.M. (1993) Competitive inhibition of abscisic acid-regulated gene expression by stereoisomeric acetylenic analogs of abscisic acid. *Plant Physiol.* **101**, 469-476.

137. Robertson, A.J., Reaney, M.J.T., Wilen, R.W., Lamb, N., Abrams, S.R. and Gusta, L.V. (1994) Effects of abscisic acid metabolites and analogs on freezing tolerance and gene expression in bromegrass (*Bromus inermis* Leyss) cell cultures. *Plant Physiol.* **105**, 823-830.

138. Hill, R.D., Liu, J.-H., Durnin, D., Lamb, N., Shaw, A. and Abrams, S.R. (1995) Abscisic acid structure-activity relationships in barley aleurone layers and protoplasts. *Plant Physiol.* **108**, 573-579.

139. Blake, T.J., Tan, W.T. and Abrams, S.R. (1990) Antitranspirant action of abscisic acid and ten synthetic analogs in black spruce. *Physiol. Plant.* **80**, 365-370.

140. Blake, T.J., Bevilacqua, E., Hunt, G.A. and Abrams, S.R. (1990) Effects of abscisic acid and its acetylenic alcohol on dormancy, root development and transpiration in three conifer species. *Physiol. Plant.* **80**, 371-378.

141. Dong, J.-Z., Pilate, G., Abrams, S.R. and Dunstan, D.I. (1994) Induction of a wheat *Em* promoter by ABA and optically pure ABA analogs in white spruce (*Picea glauca*) protoplasts. *Physiol. Plant.* **90**, 513-521.

142. Rose, P.A., Abrams, S.R. and Shaw A.C. (1992) Synthesis of chiral acetylenic analogs of the plant hormone abscisic acid. *Tetrahedron: Asymmetry* **3**, 443-450.

143. Flores, A., Grau, A., Laurich, F. and Dörffling, K. (1988) Effect of new terpenoid analogues of abscisic acid on chilling and freezing resistance. *J. Plant Physiol.* **132**, 362-369.

144. Wen, C.-S., Tseng, M.-J. and Lin, C.-H. (1993) Effect of LAB 173711, an ABA analogue, on low temperature resistance of suspension cultured sugarcane cells. *J. Plant Physiol.* **141**, 240-244.

145. Flores, A. and Dörffling, K. (1990) A comparative study of the effects of abscisic acid analogues on plant physiological processes. *J. Plant Growth Regul.* **9**, 133-139.

146. Schubert, J., Röser, K., Grossmann, K., Sauter, H. and Jung, J. (1991) Transpiration-inhibiting abscisic acid analogs. *J. Plant Growth Regul.* **10**, 27-32.

147. Van der Meulen, R.M., Heidekamp, F., Jastorff, B., Horgan, R. and Wang, M. (1993) Effects of abscisic acid analogues on abscisic acid-induced gene expression in barley aleurone protoplasts: relationship between structure and function of the abscisic acid molecule. *J. Plant Growth Regul.* **12**, 13-19.

148. Devine, M.D., Harren, J.C., Abrams, S.R. and Gusta, L.V. (1995) Effect of drought stress, abscisic acid, and abscisic acid analogues on the efficacy of diclofop-methyl and tralkoxydim. *J. Plant Growth Regul.* **14**, 77-84.

149. Lei, B., Abrams, S.R., Ewan, B. and Gusta, L.V. (1994) Achiral cyclohexadienone analogues of abscisic acid: synthesis and biological activity. *Phytochemistry* **37**, 289-296.

150. Gusta, L.V., Ewan, B. and Reaney, M.J.T. (1992) The effect of abscisic acid and abscisic acid metabolites on the germination of cress seed. *Can. J. Bot.* **70**, 1550-1555.
151. Roberts, D.L., Heckman, R.A., Hege, B.P. and Bellin, S.A. (1968) Synthesis of (*RS*)-abscisic acid. *J. Org. Chem.* **33**, 3566-3569.
152. Wang, T.L., Cook, S.K., Francis, R.J., Ambrose, M.J. and Hedley, C.L. (1987) An analysis of seed development in Pisum sativum. *J. Exp. Bot.* **38**, 1921-1932.
153. Horgan, R., Neill, S.J., Walton, D.C. and Griffin, D. (1983) Biosynthesis of abscisic acid. *Biochem. Soc. Trans.* **11**, 553-557.
154. Penglis, A.A.E. (1981) Fluorinated carbohydrates. *Adv. Carbohydr. Chem. Biochem.* **38**, 195-285.
155. Nelson, L.A.K., Shaw, A.C. and Abrams, S.R. (1991) Synthesis of (+)-, (-)- and (±)-7'-hydroxyabscisic acid. *Tetrahedron* **47**, 3259-3270.
156. Tewson, T.J. and Welch, M.J. (1978) New approaches to the synthesis of 3-deoxy-3-fluoro-D-glucose. *J. Org. Chem.* **43**, 1090-1092.
157. Rozen, S., Faust, Y. and Ben-Yakov, H. (1979) A new method for fluorination of sterols. *Tetrahedron Lett.* **20**, 1823-1826.
158. Bannai, K., Toru, T., Oba, T, Tanaka, T., Okamura, N., Watanabe, K., Hazato, A. and Kurozumi, S. (1983) Synthesis of chemically stable prostacyclin analogs. *Tetrahedron* **39**, 3807-3819.
159. Juaristi, E. (1991) Stereochemistry of organic reactions. In *Introduction to stereochemistry and conformational analysis*. John Willey & Sons, New York, pp. 72-90.
160. Fersht, A.R. (1987) The hydrogen bond in molecular recognition. *Trends Biochem. Sci.* **12**, 301-304.
161. Fersht, A.R., Shi, J.-P., Knill-Jones, J., Lowe, D.M., Wilkinson, A.J., Blow, D.M., Brick, P., Carter, P., Waye, M.M.Y. and Winter, G. (1985) Hydrogen bonding and biological specificity analysed by protein engineering. *Nature* **314**, 235-238.
162. Oritani, T., Yamashita, K. and Oritani, T. (1985) Metabolism of (±)-(2*Z*,4*E*)-α-ionylideneacetic acid and its 1'-hydroxyl analog in plants. *J. Pesticide Sci.* **10**, 535-540.
163. Toromanoff, E. (1980) Dynamic stereochemistry of the 5-,6- and 7-menbered ring using the torsion angle notation. *Tetrahedron* **36**, 2809-2931.
164. Ueda, H. and Tanaka, J. (1977) The crystal and molecular structure of *dl*-2-*cis*-4-*trans*-abscisic acid. *Bull. Chem. Soc. Jpn* **50**, 1506-1509.
165. Schmalle, V.H.W., Klaska, K.H. and Jarchow, O. Die Kristall- und Molekülstruktur von (*R,S*)-*cis,trans*-Abscisinsäure: 5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentadiensäure. *Acta Cryst.* B**33**, 2218-2224.
166. Willows, R.D. and Milborrow, B.V. (1993) Configuration and conformations of abscisic acid. *Phytochemistry* **34**, 233.
167. Harada, N. (1973) Absolute configuration of (+)-*trans*-abscisic acid as determined by a quantitative application of the exciton chirality method. *J. Am. Chem. Soc.* **95**, 240-242.

168. Ohloff, G., Otto, E., Rautenstrauch, V. and Snatzke, G. (1973) Chiroptische Eigenschaften einiger Trimethylcyclohexen-Derivate: Die Jonone, Irone und Abszisinsäuren. *Helv. Chim. Acta* **56**, 1874-1882.
169. Snatzke, G. (1965) Circulardichroismus—VIII. Modifizierung der Octantenregel für α,β-Ungesättigte Ketone: Theorie. *Tetrahedron* **21**, 413-419.
170. Snatzke, G. (1965) Circulardichroismus—IX. Modifizierung der Octantenregel für α,β-Ungesättigte Ketone: Transoide Enone. *Tetrahedron* **21**, 421-438.
171. Gawronski, J.K. (1982) Circular dichroism and stereochemistry of chiral conjugated cyclohexenones. *Tetrahedron* **38**, 3-26.
172. Abraham, R.J. and Lucas, M.S. (1988) Conformational analysis. Part 10. A theoretical and nuclear magnetic resonance (lanthanide-induced shift) study of the conformation of cyclohex-2-enone. *J. Chem. Soc., Perkin Trans. II*, 669-672.
173. Juaristi, E. (1991) The conformational analysis of acyclic and cycloalkanes. In *Introduction to stereochemistry and conformational analysis*. John Willey & Sons, New York, pp. 237-252.
174. Juaristi, E. (1991) Application of dynamic NMR spectroscopy in conformational analysis. In *Introduction to stereochemistry and conformational analysis*. John Willey & Sons, New York, pp. 253-270.
175. The auther measured ^{1}H NMR of (±)-ABA in acetone-d_6 at 185-300 K. According to this preliminary experiment, the 5'-protons broadened as the temperature became lower until they changed from the sharp AB quartet into the broad doublet at 250 K, and they sharpened again as the temperature became low further until the small signals began to appear at 220 K. At 185 K seven minor ^{1}H signals were observed. These signals seems to correspond to the 6'-methyl (8'- or 9'-protons), 7'- or 6-protons, 5'-proton (*pro-R* or *pro-S*), 1'-hydroxyl proton, 3'-proton, 5-proton and 4-proton of a minor conformer, probably the half-chair **HC2** with the side chain pseudo-equatorial. If the coalescence temperature is 250 K, the activation energy for ring inversion is calculated to be about 11 kcal mol^{-1}.[174] The NOE experiments at low temperatures will be necessitated to prove that the minor signals derived from the half-chair **HC2**.
176. Abrams, S.R. and Milborrow, B.V. (1991) Synthesis and biological activity of allenic analogues of abscisic acid. *Phytochemistry* **30**, 3189-3195.
177. Milborrow, B.V. and Abrams, S.R. (1993) Metabolism of allenic analogues of abscisic acid. *Phytochemistry* **32**, 827-832.
178. de Meijere, A. (1979) Bonding properties of cyclopropane and their chemical consequences. *Angew. Chem. Int. Ed. Engl.* **18**, 809-826.
179. Wong, H.N.C., Hon, M.-Y., Tse, C.-W., Yip, Y.-C., Tanko, J. and Hudlicky, T. (1989) Use of cyclopropanes and their derivatives in organic synthesis. *Chem. Rev.* **89**, 165-198.
180. Dolata, D.P. and Spina, D.R. (1990) Theoretical studies of the conformational control of additions to bicyclo[m.1.0]alkan-2-ones. Application and validation of the wizard technique. *Tetrahedron* **31**, 6811-6814.
181. Dolata, D.P., Spina, D.R. and Mash, E.A. (1992) Systematic search of transition state conformations. *Tetrahedron* **33**, 6571-6574.

182. Oberhammer, H. and Bauer, S.H. (1969) Structures and conformations of the cyclohexadienes. *J. Am. Chem. Soc.* **91**, 10-16.

183. Allinger, N.L. and Sprague, J.T. (1972) Conformational analysis. LXXXIV. A study of the structures and energies of some alkenes and cycloalkanes by the force field method. *J. Am. Chem. Soc.* **94**, 5734-5747.

184. Jeffrey, G.A. (1988) The structure of 1,4-cyclohexadiene at 153 K. *J. Am. Chem. Soc.* **110**, 7218-7219.

185. Corey, E.J. and Chaykovsky, M. (1965) Dimethyloxosulfonium methylide ($(CH_3)_2SOCH_2$) and dimethylsulfonium methylide ($(CH_3)_2SCH_2$). Formation and application to organic synthesis. *J. Am. Chem. Soc.* **87**, 1353-1364.

186. Mayer, H.J., Rigassi, N., Schwieter, U. and Weedon, B.C. (1976) Synthesis of abscisic acid. *Helv. Chim. Acta* **59**, 1424-1427.

187. Corey, E.J., Gilman, N.W. and Ganem, B.E. (1968) New methods for the oxidation of aldehydes to carboxylic acids and estesrs. *J. Am. Chem. Soc.* **90**, 5616-5617.

188. The inhibitory activity of PA in the α-amylase induction in barley seeds has been reported also by other groups (see ref. 84 and references cited therein). Recently, it has been reported that PA can be converted into 8'-hydroxy-ABA inversely under the acidic condition, and that 8'-hydroxy-ABA shows the equivalent activity to ABA (see ref. 56). Therefore, there might be an unknown mechanism of such back isomerization in barley seeds, although of course it may be explained more ordinarily by the difference in steric requirements of the receptor among species or tissues.

List of Publications

Original Articles

1) Todoroki, Y., Hirai, N., Koshimizu, K. (1994) 8'- and 9'-Methoxyabscisic acids as antimetabolic analogs of abscisic acid. *Biosci. Biotech. Biochem.* **58** (4), 707-715.

2) Todoroki, Y., Hirai, N., Koshimizu, K. (1995) 8',8'-Difluoro- and 8',8',8' trifluoroabscisic acids as highly potent, long-lasting analogues of abscisic acid. *Phytochemistry* **38** (3), 561-568.

3) Todoroki, Y., Hirai, N., Ohigashi, H. (1995) Synthesis, biological activity and metabolism of (*S*)-(+)-3'-fluoroabscisic acid. *Tetrahedron* **51** (25), 6911-6926.

4) Todoroki, Y., Hirai, N., Ohigashi, H. (1995) Synthesis and biological activity of 1'-deoxy 1'-fluoro- and 8'-fluoroabscisic acids. *Phytochemistry* **40** (3), 633-641.

5) Nakano,S., Todoroki, Y., Hirai, N. and Ohigashi, H. (1995) Synthesis and biological activity of 7'-, 8'-, and 9'-alkyl analogues of abscisic acid. *Biosci. Biotech. Biochem.* **59** (9), 1699-1706.

6) Todoroki, Y., Nakano, S., Hirai, N. and Ohigashi, H. (1996) Ring conformational requirement for biological activity of abscisic acid probed by the cyclopropane analogues., *in preparation*.

Review

1) Todoroki, Y. and Nirai, N. (1995) *Kagaku to Seibutsu* **33** (2), 122-130 (in Japanese).