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Electrolytic reduction of abscisic acid methyl ester and its free acid

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ABSTRACT

Abscisic acid (ABA, **1**), a plant hormone, has electrophilicity derived almost entirely from the side-chain, 3-methylpenta-2,4-dienoic acid. The electrochemical property of ABA was investigated by analysis of its cathodic reaction. ABA methyl ester (**1**-**Me**) was reduced at a peak potential of −1.6 V to give a unique and unstable bicyclic compound (**5**-**Me**) as a major product at pH 3 and 7. This finding showed that an electron was absorbed in the conjugated dienecarboxyl group, and that C-5 with a high electron density attacked C-2′ through an intramolecular nucleophilic addition. At pH 10, in addition to **5**-**Me**, a compound **4**-**Me** was formed by isomerization of **5**-**Me** under alkaline conditions. For a cathodic reaction of ABA at pH 3 and 7, compound **5** was a major product as well as in the case of ABA methyl ester. However, at pH 10, a dimer (**6**) with an epoxy group, 1′-deoxy-ABA (**7**) and other compounds were formed instead of compounds **4** and **5**. Compounds **4** and **5** were biologically inactive, suggesting the importance of the electrophilic side-chain of ABA for biological activity.

1. Introduction

Abscisic acid (ABA, **1**), a sesquiterpenoid, is a plant hormone that induces physiological adaptation to environmental stresses such as drought and low temperature (Hirai, 2010). The biological response of ABA (**1**) has recently been shown to be regulated by a combination of a receptor protein, a negative regulator, and a positive regulator (Ma et al., 2009; Park et al., 2009; Umezawa et al., 2010). ABA (**1**) has a conjugated dienecarboxyl group, a tertiary hydroxyl group and an enone group as functional groups. The C-1′ of ABA (**1**) is chiral, and natural ABA used in this study is the *S*-(+)-isomer. These give ABA (**1**) its characteristic physical and chemical properties. The electrophilicity is one of the properties of ABA (**1**).

The electrophilicity of ABA (**1**) is derived almost entirely from the side-chain, 3-methylpenta-2,4-dienoic acid, which is supported by the following evidence. ABA methyl ester (**1**-**Me**) it shows high sensitivity to an electron capture detector in a gas chromatograph. An electron capture detector can detect 1 pg of ABA methyl ester (**1**-**Me**), while its detection limit by a flame ionization detector is 1 ng (Seely and Powell, 1970). This means that ABA methyl ester **1**-**Me** absorbs an electron to form an anion radical. In mass spectrometry, it gives a more stable molecular ion at a negative ion mode than that at a positive ion mode. In chemical ionization mass spectrometry, the intensity ratio of an $[M]^-$ ion to an $[M]^+$ ion is about 20:1 (Netting et al., 1988). Terem and Utley reported that in electrolysis ABA methyl ester (**1**-**Me**) was irreversibly reduced with two electrons at a cathode to give a bicyclic product (**2**) (Terem and Utley, 1979). The formation mechanism of the bicyclic product they proposed is as follows: ABA methyl ester (**1**-**Me**) absorbs an electron to form an anion radical, and C-2 with a high electron density attacks C-2′ through an intramolecular nucleophilic addition; after addition of another electron and two protons to this intermediate, the bicyclic product is formed.

The electrophilicity of ABA (**1**) might be involved in its binding to a receptor protein and other ABA-binding proteins for expression of the biological activity of ABA (**1**). Before investigating the effect of the electrophilicity of ABA (**1**) on biological activity, Terem and Utley's experiment was investigated herein, where it was found that electrolysis of ABA methyl ester (**1**-**Me**) gave a product with a structure different from that reported by Terem and Utley. This finding led us to re-examination of the electrochemical reaction of ABA methyl ester (**1**-**Me**). This paper describes the electrochemical reaction products of ABA methyl ester (**1**-**Me**) and ABA (**1**) and discusses the reaction mechanism. The electrochemical reaction of phaseic acid (**3**), a natural metabolite of ABA (**1**) (Hirai, 2010), and the biological activity of those products are also reported. The numbering system of ABA (**1**) shown in the structure is used in this paper.

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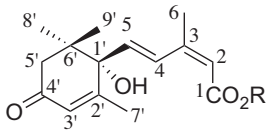

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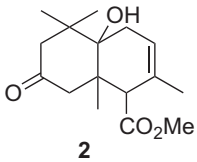
1-Me: R=Me



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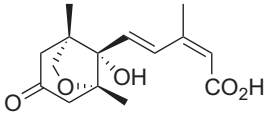

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2. Results and discussion

2.1. Cathodic reaction of ABA methyl ester (**1-Me**)

2.1.1. Cyclic voltammetry

ABA methyl ester (**1-Me**) was electrolyzed at a cathode under the same condition as that of Terem and Utley who used CH_3CN containing 0.1 M $(n\text{-}Bu)_4NOAc$/HOAc as catholyte (Terem and Utley, 1979). However, no significant product was formed under those conditions. A proton donor should be necessary for cathodic reduction, so water was added to the solution. When **1-Me** was reduced in an aqueous CH_3CN at a cathode without pH control, formation of a major product (**4**) was observed. The yield of **4** increased as the water content increased, and reached 68% at 80% of water content. The methyl ester (**4-Me**) of **4** was also detected in a yield of 5% at 80% of water content. The pH of the cathode solution after electrolysis was 12, suggesting that the initial product of electrolysis was **4-Me**, and that **4-Me** was hydrolyzed under alkaline condition to give **4** as the major product. The CH_3CN used by Terem and Utley might contain water, and the pH might be controlled although the detail is unknown. The structure of compound **4-Me** seemed to be the same as that of compound **2** since the spectral data of **4-Me** were similar to those of **2** (Terem and Utley, 1979), but the structure of **4-Me** was completely different from that of **2** as shown later. Electrolysis of **1-Me** was then re-examined under pH-controlled conditions, and the electrochemical reaction mechanism of **1-Me** at a cathode.

Cyclic voltammetry of **1-Me** with a glassy carbon cathode was done in 20% CH_3CN aqueous buffer solutions at pH 3, 7 and 10. Fig. 1 shows the voltammogram measured at pH 7 at a scan rate of 100 mV/s. On the cathodic scan, a reduction peak was observed at E_p − 1.61 V with a shoulder peak at E_p − 1.76 V, and an oxidation peak was not observed upon reversal of the direction of potential scan. This was almost coincident with the result of Terem and Utley. The major peak would correspond to reduction of the side-chain, and the shoulder peak to reduction of the enone group. The voltammograms at other pHs were similar to those at pH 7. The major irreversible reduction peaks were observed at E_p values of −1.59 V and −1.87 V at pH 3 and pH 10, respectively. These results indicated that **1-Me** was reduced irreversibly in both acidic and alkaline solutions.

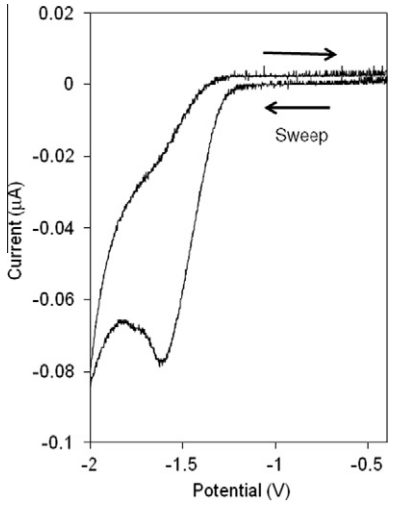


Fig. 1. Voltammogram of ABA methyl ester (**1-Me**) at pH 7.0. Substrate: 5 mM **1-Me**; solution: 50 mM phosphate buffer of pH 7 containing CH_3CN–H_2O (1:4, v/v); supporting electrolyte: 0.1 M $(C_2H_5)_4NClO_4$; working electrode: glassy carbon; counter electrode: Pt; reference electrode: Ag/AgCl; salt bridge: agar containing KCl; sweep speed: 100 mV/s; sweep range: −0.4 to −2.0 V.

2.1.2. Products of the cathodic reaction

To analyze the reaction product, **1-Me** was electrolyzed on a large scale using a mercury pool as a cathode to avoid coating the electrode surface with reaction products. Compound **1-Me** (0.125 mmol) was electrolyzed in 20% CH_3CN buffer solutions of pH 3, 7 and 10 at −2.0 V until the current fell to the background level. After electrolysis, the electrolysis solutions in the cathode compartment were analyzed by HPLC. The composition of the cathodic reaction products is shown in Table 1. At pH 3 and 7, **5-Me** was a major product in 83% and 93% yield, respectively, and, at pH 10, **4-Me** was formed in 44% yield in addition to **5-Me** of 27% yield. Residual amounts of **1-Me** were small, and minor products were observed at the three pHs. The total electric charge at pH 3, 7 and 10 were 22.7 C, 24.1 C and 20.4 C, respectively. If **1-Me** is reduced with two electrons, those total electric charges correspond to 94%, 102%, and 85%, respectively, of the electric charge to reduce 0.125 mmol of **1-Me**. This finding indicated that the cathodic reaction proceeds almost quantitatively. Compounds **4-Me** and **5-Me** were isolated from the cathodic solutions to elucidate their structures. Compound **5-Me** was unstable, and gradually decomposed to several unknown compounds on heating during concentration. Concentration of **5-Me** was conducted at a temperature lower than 30 °C within a short time after extraction of compound **5-Me** with EtOAc from the aqueous solution.

Compound **5-Me** had −44° as a specific optical rotation value. The mass spectrometry of **5-Me** showed a molecular ion at m/z 280, and with high resolution setting gave a molecular ion at m/z 280.1675 corresponding to a molecular formula of $C_{16}H_{24}O_4$. The molecular formula indicated that **5-Me** had two more protons than **1-Me**, being coincident with two-electron reduction. Compound **5-Me** showed a weak absorption maximum at 206 nm ($\log\varepsilon$ 3.37) in the UV spectrum, suggesting that there is no conjugated double

Table 1
Composition of the cathodic reaction products of ABA methyl ester (**1-Me**) at three pHs.

pH	Products (%)			
	5-Me	**4-Me**	**1-Me**[a]	Others[b]
3	83	0[c]	10	7
7	93	0	1	6
10	27	44	18	11

[a] Recovered **1-Me**.
[b] Unidentified products.
[c] Zero means "not detected".

bond. In the IR spectrum, the absorption at 1722 and 1734 cm^{-1} was indicative of non-conjugated carbonyl and ester groups, respectively, and the absorption at 3736 cm^{-1}suggested the presence of a hydroxyl group. The ^{1}HNMR spectrum of **5**-**Me** showed signals assignable to three tertiary methyl groups at δ 1.13, 1.15 and 1.19 ppm, one methyne proton at δ 1.67 ppm (d, J = 7.3 Hz), a methyl group on a double bond at δ 1.76 ppm (d, J = 1.0 Hz), a hydroxyl group at δ 2.04 ppm, four methylene protons adjacent to a carbonyl group at δ 2.22 ppm (2H, s), 2.60 ppm (1H, d, J = 19.6 Hz) and 2.68 ppm (1H, d, J = 19.6 Hz), two methylene protons adjacent to a methoxycarbonyl group and a double bond at δ 3.09 ppm(s), carboxymethyl protons at δ 3.69 ppm, and one olefin proton at δ 5.20 ppm (dd, J = 7.3 and 1.0 Hz). The assignment of the signal at δ 2.04 ppm to a hydroxyl group was confirmed by disappearance of the signal with addition of D_2O. The ^{1}HNMR spectrum showed that three olefin protons among the four olefin protons, H-2, 4, 5 and 3′, of **1**-**Me** changed to two methylene and one methyne protons, and that one methyl group, C-6 or C-7′, of **1**-**Me** shifted to the high magnetic field. This suggested that two hydrogen atoms were added to a double bond at C-2 or C-4 or C-2′of **1**-**Me**. The four methylene protons at δ 2.22, 2.60 and 2.68 ppm could be assigned to H-3′ and H-5′ adjacent to a carbonyl group at C-4′, suggesting that the double bond at C-2′ was saturated. This meant that the H-7′ signal should shift to a higher magnetic field, and appear as a secondary methyl group. However, signals assignable to H-2′ and a secondary methyl group were not observed. C-2′ would be quaternary since only tertiary methyl signals were observed at δ 1.13, 1.15 and 1.19 ppm. This suggested that the signal at δ 1.76 ppm was assigned to H-6, meaning presence of a double bond at C-2 or C-3. This double bond should be adjacent to a carbon having a methyne proton since the olefin proton was coupled with a methyne proton. If the double bond is located at C-3, the olefin proton could be assignable to H-4, the methyne proton to H-5, and the two methylene protons at δ 3.09 ppm that did not show any coupling with other protons could be assigned to H-2. This assignment inevitably meant that C-5 bonded to C-2′ to form a cyclopropyl ring. The relationship between C-5, 1′ and 2′ was examined by the ^{13}CNMR, HMQC and HMBC spectra of **5**-**Me**.

The ^{13}C NMR spectrum of **5**-**Me** showed 16 signals, and the resonances of C-2, 4, 5, 6, 3′, 5′, 7′, 8′, 9′ and a carboxymethyl carbon bonding to the protons were tentatively assigned by the HMQC spectrum. The other carbon signals were assignable to C-1, 3, 1′, 2′, 4′ and 6′ based on their chemical shifts. In the HMBC spectrum, the methyne proton (H-5) showed cross-peaks not only with C-3, 2′ and C-6′, but also with C-3′ and C-7′, and the olefin proton (H-4) showed cross-peaks with C-2′ in addition to C-2, C-6 and C-1′ (Fig. 2). These findings confirmed that C-5, C-1′ and C-2′consisted of a cyclopropyl ring. The configuration at C-5 and assignment of the other protons signals were determined by NOESY. The NOESY spectrum of **5**-**Me** showed cross-peaks between the H-5 signal, and the methyl proton signal at δ 1.13 ppm and the H-6 signal at

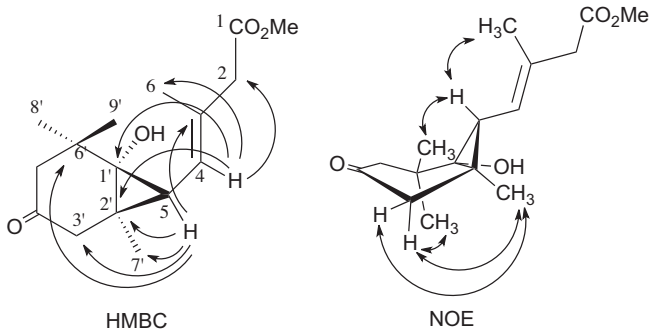


Fig. 2. Characteristic correlations in HMBC and NOESY spectra of compound **5**-**Me**. The NOE correlation between H-5′, and H-8′ and H-9′ is not shown to avoid complication.

δ 1.76 ppm (Fig. 2). If the configuration at C-5 was *S*, there would be no cross-peak between H-5 and any methyl groups, so those cross-peaks indicated that the absolute configuration at C-5 was *R*. This configuration indicated also that the methyl proton signal at δ 1.13 ppm was H-9′. The methylene signal at δ 2.22 ppm showed cross peaks with the methyl signals at δ 1.13 ppm and 1.19 ppm, the methylene signal cross peaks at δ 2.60 ppm with the methyl signals at δ 1.15 and 1.19 ppm, and the methylene resonance at δ 2.68 ppm a cross-peak with the methyl signal at δ 1.15 ppm. These findings finally assigned the signals at δ 1.13, 1.15, 1.19, 2.22, 2.60 and 2.68 ppm to H-9′, H-7′, H-8′, H5′, H-3′$_{pro\text{-}S}$ and H-3′$_{pro\text{-}R}$, respectively. A cross-peak between H-4 and H-6 was not observed, showing that the double bond at C-3 was *E*. Thus, the structure of compound **5**-**Me** was identified as (−)-(*E*)-methyl 4-((1*S*,6*R*,7*R*)-1-hydroxy-2,2,6-trimethyl-4-oxobicyclo[4.1.0]heptan-7-yl)-3-methylbut-3-enoate. Instability of **5**-**Me** on heating could be caused by the angle strain of the cyclopropyl group. The formation mechanism of **5**-**Me** will be discussed later.

The mass spectrum of **4**-**Me**showed a molecular ion at m/z 280, and its molecular formula was determined as $C_{16}H_{24}O_4$ by high resolution mass spectrometry, indicating that **4**-**Me** had two more protons than **1**-**Me** as well as **5**-**Me**. Compound **4**-**Me** showed a weak UV absorption at 204 nm, suggesting that **4**-**Me** did not have conjugated double bonds. In the IR spectrum of **4**-**Me**, absorption bands at 1712 and 1736 cm^{-1} corresponding to carbonyl and methoxycarbonyl groups, respectively, were observed, and an absorption at about 3500 cm^{-1}corresponding to a hydroxyl group was absent. The ^{1}HNMR spectrum was similar to that of **5**-**Me**, except for the disappearance of the methyne proton (H-5) signal and appearance of methylene proton signal at δ 2.07 ppm (1H, dd, J = 14.0 and 8.9 Hz) and 2.55 ppm (1H, dd, J = 14.0 and 7.3 Hz). Coupling of the methylene protons with each other and with an olefin proton assignable to H-4 suggested that the tertiary C-5 of **5**-**Me** was changed to a secondary carbon in **4**-**Me**. The ^{13}CNMR spectrum

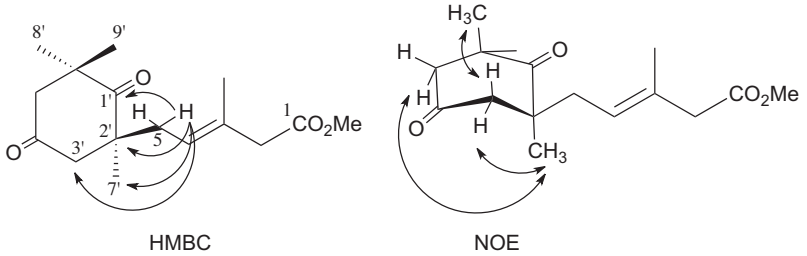
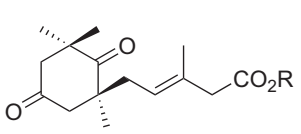
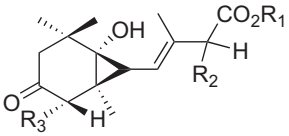


Fig. 3. Characteristic correlations in HMBC and NOESY spectra of a stable conformation of compound **4**-**Me**. The HMBC correlation between H-5, and C-3and C-4, and the NOE correlation between H-7′ and H-5 are not shown to avoid complication.



4: R=H
4-Me: R=Me



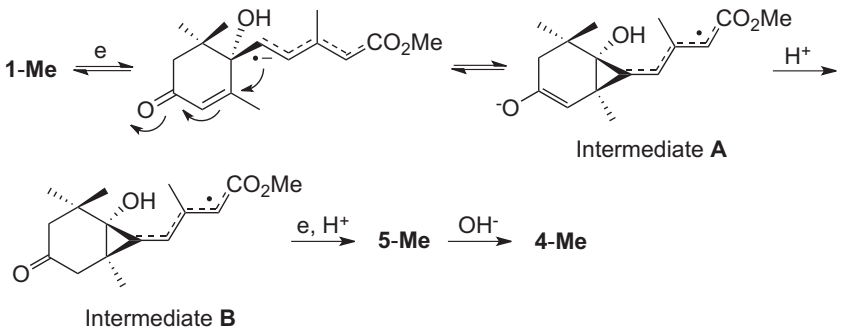
5: R_1=R_2=R_3=H
5-Me: R_1=Me, R_2=R_3=H
5-Me-d_2:R_1=Me, R_2=D, R_3=D

of **4**-**Me** was also similar to that of **1**-**Me**, but a carbon bonding to a hydroxyl group was not observed and another carbonyl signal was observed at δ 215.8 ppm in addition to a carbonyl signal at δ 208.8 ppm assignable to C-4′. This finding suggested that the hydroxyl group at C-1′ was changed to a carbonyl group. In the HMBC spectrum of **4**-**Me**, theH-5 signal at δ 2.55 showed cross-peaks with C-3, C-4, C-2′, C-3′ and C-7′, and the other H-5 signal at δ 2.07 ppm showed a cross-peak with C-1′ in addition to those cross-peaks, but both H-5 signals did not show a cross peak with C-6′ (Fig. 3). These findings indicated that the **4**-**Me** had a carbonyl group at C-1′ and the side-chain at C-2, indicating that **4**-**Me** was an isomer of **5**-**Me**. Since **4**-**Me** seemed to be formed by opening the C-5-C1′ bond of **5**-**Me**, the absolute configuration at C-2′ would be *S*. Assignment of prochiral H-3′ and H-5′, H-8′ and H-9′ signals in the ^{1}HNMR was elucidated by analysis of the NOESY spectrum (Fig. 3). In the NOESY spectrum of **4**-**Me**, H-7′ showed cross-peaks with the both H-5, one H-3′at δ 2.46 ppm, and one H-5′at δ 2.58 ppm. Stable geometries of **4**-**Me** were obtained by theoretical calculation using density functional theory (Gaussian 09, 2009). The stable conformation of the cyclohexanone ring of **4**-**Me** is in its twist boat forms rather than half chair forms due to the 1,3-diaxial interactions between the side-chain and the methyl group or between the methyl groups. The most stable conformer was a twist boat form with the equatorial side-chain and the C-8′ methyl group, and the axial C-7′ and C-9′ methyl groups (Fig. 3).The NOE cross peaks observed are consistent with the twist boat form **A** where H-3′$_{pro\text{-}S}$ and H-5′$_{pro\text{-}R}$ are close to H-7′. This meant that H-3′ showing a cross-peak with H-7′ was *pro-S*, and another H-3′at δ 2.86 ppm inevitably was *pro-R*, and that H-5′ showing a cross-peak with H-7′ was assigned to *pro-R*, and H-5′ (δ 2.61 ppm) was *pro-S*. Among the two methyl signals at δ 1.14 and 1.20 ppm, the former one showed an NOE cross-peak with the H-3′$_{pro\text{-}R}$ signal. This relationship could be observed between H-9′ and H-3′$_{pro\text{-}R}$ in the twist boat form A also, so the methyl signal at δ 1.14 ppm was H-9′, and another one was inevitably assigned to H-8′.Thus, **4**-**Me** was identified as (*S*,*E*)-methyl 3-methyl-5-(1,3,3-trimethyl-2,5-dioxocyclohexyl)pent-3-enoate.

2.1.3. Cathodic reaction mechanism

Compound **5**-**Me** was a reduced product of **1**-**Me** formed by addition of two electrons and two protons. The structure of **5**-**Me** suggested that two protons derived from water were added one to each of C-2 and C-3′. To confirm the position of added protons, **1**-**Me** was electrolyzed at a cathode in D_2O containing 20% CH_3CN at pH 7, and compound **5**-**Me**-***d*$_2$** was isolated from the reaction solution after electrolysis. The mass spectrum of **5**-**Me**-***d*$_2$** showed molecular ions $[Md_2]^+$ at *m*/*z* 282, $[Md_1]^+$ at *m*/*z* 281 and $[Md_0]^+$ at *m*/*z* 280, being consistent with incorporation of two deuterium atoms. The ^{1}HNMR spectrum of **5**-**Me**-***d*$_2$** showed decrease in the areas, and change of the shape for the H-2 and H-3′ signals. The H-2 signal consisted of two singlets corresponding to –CH_2– and –CDH– with 1.12H of the signal area, indicating that 0.88H of 2H at C-2 was substituted with deuterium. The H-3′ signal consisted of two doublets corresponding to –CH_2–, and two singlets corresponding to –$CDH_{pro\text{-}S}$– and –$CDH_{pro\text{-}R}$– with 0.54H, 0.14H and 0.59H of the signal area, respectively. This indicated that 73% of 2H at C-3′, 59% of H-3′$_{pro\text{-}S}$ and 14% of H-3′$_{pro\text{-}R}$, were substituted with deuterium. The higher deuterium content at H-3′$_{pro\text{-}S}$ than that at H-3′$_{pro\text{-}R}$ showed that in the intermediate A, addition of a proton to C-3′ from the *si* (lower) face was preferable to that from the *re* (upper) face to form the intermediate B (Scheme 1). Addition of a proton to C-3′ from the *re* face seemed to be sterically hindered by the cyclopropyl group which probably adopts pseudo-axial orientation. The deuterium content at C-3′ lower than that at C-2 suggested that deuterium at C-3′was partially lost through enolization of **5**-**Me**-***d*$_2$** during isolation. This experiment confirmed that two protons from water were introduced almost at H-2 and H-3′$_{pro\text{-}S}$.

Based on the above results, the formation mechanism of **4**-**Me** and **5**-**Me** from **1**-**Me** at a cathode is proposed as shown in Scheme 1. The reaction would progress via the following steps: the first step of the reaction is addition of one electron to the side-chain of **1**-**Me**, then the electron localizes at C-5, and C-5 attacks C-2′ with a low electron density by the intramolecular conjugated addition to form the enol intermediate A with the cyclopropyl ring; one proton from water is added to C-3′ of the



Scheme 1. Formation mechanism of compounds **5**-**Me** and **4**-**Me** from ABA methyl ester (**1**-**Me**) at a cathode.

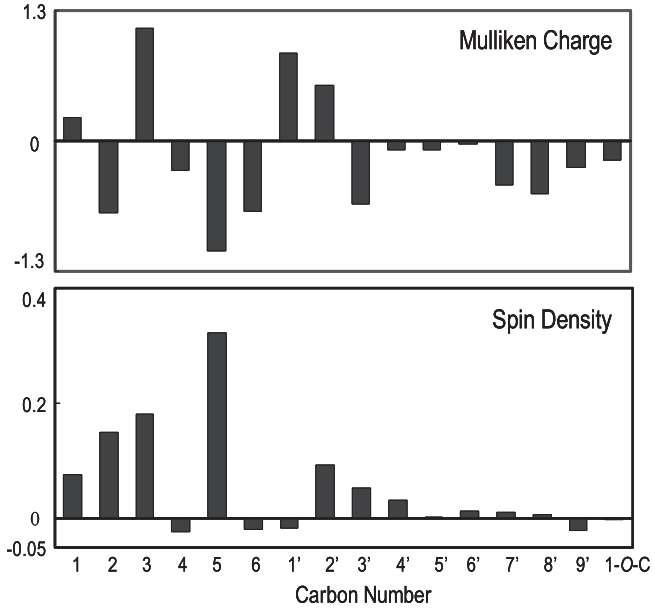


Fig. 4. Mulliken charge and spin density of individual carbons of ABA methyl ester (**1**-**Me**) anion radical.

Table 2
Composition of the cathodic reaction products of ABA (**1**) at three pHs.

pH	Products (%)				
	5	**6**	**7**	**1**[a]	Others[b]
3	86	0[c]	0	13	1
7	75	7	0	18	0
10	0	7	6	39	48

[a] Recovered **1**.
[b] Unidentified products.
[c] Zero means "not detected".

intermediate **A** from the *si* face to form the intermediate **B** as described above; finally, addition of one electron and one proton to C-2 results in formation of **5**-**Me**. Compound **4**-**Me** would be formed by isomerization of **5**-Me. Actually **5**-**Me** can be changed easily to **4**-**Me** under alkaline conditions. Under alkaline conditions, dissociation of the hydroxyl group at C-1′ of **5**-**Me** would trigger the formation of a carbonyl group accompanied with fission of the C-5-C-1′ bond, and addition of another proton to C-5 would give **4**-**Me**.

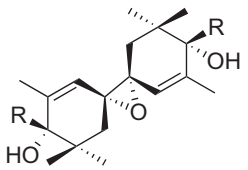
No product was detected having the structure of **2** in the cathodic solutions after electrolysis of **1**-**Me**. Furthermore, the spectroscopic data of **2** reported by Terem and Utley were very similar to those of **5**-**Me**. This finding strongly suggested that Terem and Utley incorrectly identified **5**-**Me** as **2**. Compound **4**-**Me** had also the spectroscopic data similar to those reported for **2**, but the IR spectrum data reported for **2** showed the presence of a hydroxyl group, so **4**-**Me** may have been excluded from the products obtained by Terem and Utley. The difference of the formation mechanism between **5**-**Me** and **2** is the localization of the first electron added to the side-chain. Our mechanism for the formation of **5**-**Me** postulates that the first electron localizes at C-5 resulting in the intramolecular nucleophilic addition of C-5 to C-2′, while the formation mechanism of **2** postulates that the first electron localizes at C-2 to attack C-2′. To examine the localization of the first electron, Mulliken charge and spin density at the individual carbons of **1**-**Me** anion radical were calculated using the non-empirical molecular orbital method (Gaussian 98, 1998). The result indicated that the negative Mulliken charge and the spin density at C-5 were the highest among the 16 carbons (Fig. 4). The negative Mulliken charge at C-2 was the second, and the spin density at C-2 was the third among the 16 carbons. This calculation coincides the mechanism that the first electron localized at C-5 to form compound **5**-**Me**. Formation of **2** in the cathodic reaction of **1**-**Me** is unlikely, apart from the structure problem. Thus, formation of **5**-**Me** in the cathodic reaction of **1**-**Me** has been confirmed along with its formation mechanism.

2.2. Cathodic reaction of ABA (*1*)

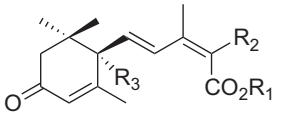
2.2.1. Cyclic voltammetry and cathodic reaction product

A cathodic reaction of **1** was examined for comparison with that of **1**-**Me**. Cyclic voltammetry of **1** was measured in 0.7% EtOH aqueous solutions of pH 3, 7 and 10 using a glassy carbon cathode. Irreversible reduction peaks were observed at E_p values of −1.65, −1.68, −1.77 V at pH 3, 7 and 10, respectively (data not shown). This result indicated that **1** was reduced irreversibly in both acidic and alkaline conditions similarly to **1**-**Me**.

To analyze and identify the cathodic reaction products of **1**, 0.068 mmol of **1**was electrolyzed at −2.0 V using a mercury pool as a cathode. The composition of the cathodic reaction products analyzed by HPLC is shown in Table 2. At pH 3 and 7, the major product was compound **5**, the yields of which were 86% and 75%, respectively. At pH 7, the minor product compound **6** was detected in a yield of 7%. The total electric charge during electrolysis at pH 3 and 7 were 17.0 C and 14.5 C, respectively, which corresponded to 107% and 96%, respectively, of electric charge necessary for two-electron reduction of **1** used. This result suggested that **1** was quantitatively reduced with two electrons and two protons on a cathode at pH 3 and 7 by the same mechanism as that for **1**-**Me**. However, at pH 10, 39% of **1** used was recovered, and the products were compounds **6** (yield 7%) and **7** (yield 6%), and unknown compounds. The lower reactivity of **1** at pH 10 than that of **1**-**Me** may be caused by dissociation of the carboxyl group, i.e. a negative charge at C-1 could suppress addition of an electron to the side-chain at the first step of the reaction through electric repulsion. Compounds **6** and **7** were isolated from the electrolysis solution in the cathode compartment after electrolysis at pH 7 and at pH 10, respectively, to identify their structure.



6: R=3-methylpenta-2,4-dienoic acid
6-Me: R=methyl 3-methylpenta-2,4-dienoate



7: R_1=R_2=R_3=H
7-*d*$_2$: R_1=H, R_2=R_3=D
7-Me-*d*$_2$: R_1=Me, R_2=R_3=D

Compound **6** was methylated by diazomethane to give a dimethyl ester **6-Me**. Its mass spectrum showed a molecular ion at *m/z* 540, and the molecular formula of **6-Me** was confirmed as $C_{32}H_{44}O_7$ by the high-resolution mass spectrometry. The ^{1}HNMR and ^{13}CNMR spectra of **6-Me** showed signals of twenty-two protons and 16 carbons, respectively, corresponding to the half number of hydrogen and carbon atoms of the molecular formula. This result suggested that **6-Me** was a symmetric dimersharing one oxygen atom. The ^{1}HNMR spectrum of **6-Me** at 294 K was similar to that of **1-Me** except for an up-field shift of H-5′ signals to δ 1.36 and 1.58 ppm, and for broadness of an H-5 signal at δ 6.08 ppm and H-5′ signals. The broad signals became sharp at 325 K, and the H-5′ signals were observed as two doublets with a coupling constant of 14.8 Hz, and the H-5 signal was also a doublet coupled with H-4. These findings suggested that the carbonyl group at C-4′of **1-Me** was reduced, and that **6-Me** had conformations which interchanged slowly at 294 K. Absence of a signal assignable to H-4′ suggested that **6-Me** was a dimer of 4′-deoxy-ABA connected with an epoxy ring. The presence of an epoxy ring was confirmed by absorption at 1245 cm^{-1}in the IR spectrum. The ^{13}CNMR spectrum showed two signals of carbon bound to oxygen (δ 74.4 and 79.3 ppm) in addition to 14 carbon signals assignable to C-1-O<u>C</u>H$_3$, C-1-C-6, C-2′, C-3′ and C-5′-C-9′. The signal at δ 79.3 ppm showed cross peaks with the H-3′, 7′, 8′ and 9′ signals in the HMBC spectrum, and was assigned to C-1′, while the signal at δ 74.4 ppm showed weak cross peaks with H-3′ and 5′. This correlation meant that the signal at δ 74.4 ppm was C-4′ consisting of an epoxy ring. Thus, compound **6** was identified as 5-[10-(4-carboxy-3-methyl-buta-1,3-dienyl)-3,10-dihydroxy-2,4,4,9,11,11-hexamethyl-13-oxa-dispiro[5.0.5.1]trideca-1,8-dien-3-yl]-3-methyl-penta-2,4-dienoic acid. The specific optical rotation of **6** showed a large positive value which would be derived from the *S*-configuration at C-1′, indicating that the steric structure of **6** was asymmetric. For the absolute configuration at C-4′ and C-4″of **6**, four combinations, (4′*R*,4″*S*), (4′*R*,4″*R*), (4′*S*,4″*R*) and (4′*S*,4″*S*), are possible, but it was not elucidated. Compound **7** was identified as (±)-1′-deoxy-ABA by comparison of its spectroscopic data with those of authentic 1′-deoxy-ABA.

2.2.2. Formation mechanism of compounds *6* and *7*

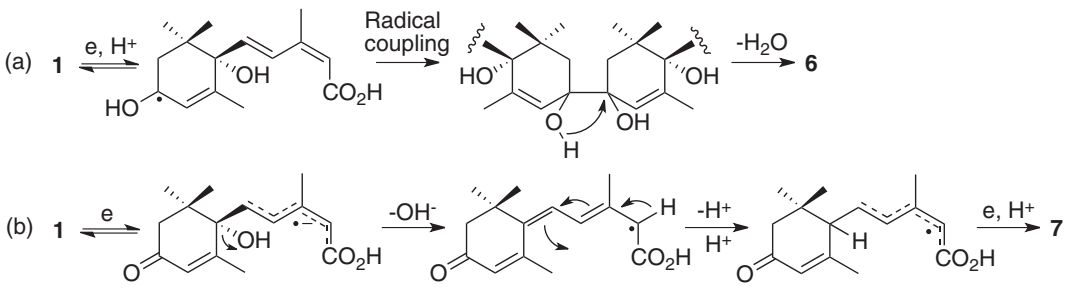
To examine the formation mechanism of **6** and**7**, **1** was electrolyzed in D_2O containing 0.7% EtOH at pH 10. The products **6** and **7-*d*$_2$** were isolated from the cathodic reaction solution. The ^{1}HNMR spectrum of **6** formed in the D_2O solution and the mass spectrum of its methyl ester did not show presence of deuterium, meaning that no deuterium from D_2O was involved in the formation of **6**. The proposed formation mechanism of **6** from **1** at a cathode is shown in Scheme 2a. One electron and one proton are added to the carbonyl carbon at C-4′ to give a radical, and then two radicals are bound at C-4′ to form a dimer. The epoxy ring forms by elimination of H_2O. The negative charge of 1-carboxyl group may cause addition of an electron at the 4′-carbonyl group.

Compound **7-*d*$_2$** gave a monomethyl ester **7-Me-*d*$_2$** on methylation. The mass spectrum of **7-Me-*d*$_2$** showed a molecular ion at *m/z* 264, indicating that the product contained two deuterium atoms. The ^{1}HNMR spectrum of **7-Me-*d*$_2$** showed that the deuterated positions were C-2 and C-1′. The individual deuteration percentages at C-2 and C-1′ calculated by the signal integral of residual H-2 and H-1′ were 89% and 65%, respectively. Introduction of deuterium at C-2 and C-1′ suggested formation mechanism of **7** from **1** at a cathode shown in Scheme 2b. First, one electron is added to the side-chain. The 1′-hydroxyl group of the first intermediate is eliminated probably due to instability derived from anion radical and negative charge of the 1-carboxyl group. Then, H-2 is eliminated and one proton from water is added to C-1′ along with migration of double bonds. Addition of a proton to C-1′ would occur from both sides of *re* and *si* faces at C-1′ since **7** was a racemic mixture. Finally, one electron and one proton from water are added to C-2 to form **7**.

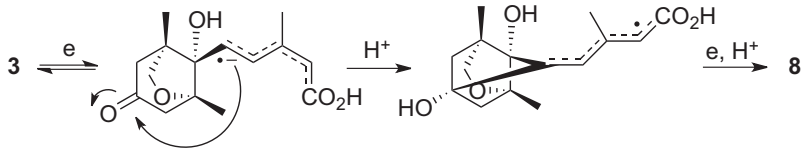
2.3. Cathodic reaction of phaseic acid (*3*)

Phaseic acid (**3**) does not possess a double bond at C-2′ in the cyclohexanone ring although it possesses the side-chain and the tertiary hydroxyl group at C-1′ as in **1**. This suggested that the nucleophilic attack of C-5 at C-2′ did not occur in phaseic acid. Cathodic reaction of **3**was examined to know the fate of phaseic acid. The cyclic voltammetry of **3** at pH 3 showed a reduction peak at E_p − 1.60 V, but did not show an oxidation peak, meaning that an irreversible reduction of **3** occurred. Compound **3** was electrolyzed at pH 3 and at −2.0 V until it disappeared, and a major product (**8**) was isolated from the electrolysis solution in the cathode compartment.

Compound **8** gave a monomethyl ester (**8-Me**) on methylation, and its high-resolution mass spectrometry showed that **8-Me** had a molecular formula of $C_{16}H_{24}O_5$, indicating that two hydrogen atoms were added to **8**. In the ^{1}HNMR spectrum of **8**, H-6, H-7′ and H-9′ were observed at δ 1.79, 1.30 and 1.09 ppm, respectively. One proton on a double bond was observed at δ 5.40 ppm as a doublet signal, and assigned to H-4 since a doublet of 1H at δ 2.94 and a singlet of 2H at δ 3.09 ppm were assignable to H-5 and H-2, respectively. H-3′ and H-5′were observed at δ 1.78 and 2.04 ppm, and δ 1.71 and 1.82 ppm, respectively, showing an up-field shift. This suggested that the 4′-carbonyl group was reduced to a hydroxyl group. In fact, the ^{13}CNMR spectrum of **8** showed a signal at δ 74.1 ppm corresponding to C-4′ bearing a hydroxyl group. However, no proton at C-4′ was observed in the ^{1}HNMR spectrum. This suggested that C-5 was bound to C-4′, and was supported by the presence of a cross-peak between C-5 and H-5′ in the HMBC spectrum of **8**. Thus, **8** was identified as (*E*)-4-(3a,5-dihydroxy-3,6a-dimethylhexahydro-2*H*-3,5-methanocyclopenta[*b*]furan-4-yl)-3-methylbut-3-enoic acid. Compound **8** was unstable, and

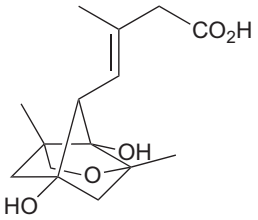


Scheme 2. Formation mechanism of compounds **6** (a) and **7** (b) from ABA (**1**) at a cathode.



Scheme 3. Formation mechanism of compound **8** from phaseic acid (**3**) at a cathode.

gradually decomposed to unknown compounds in a methanol solution at −5 °C in the dark, probably due to the strain of the tricyclic structure. The formation mechanism of **8** from **3** at a cathode is proposed in Scheme 3; an electron is added to the side-chain, and C-5 with a high electron density attacks C-4′ to form the tricyclic structure with a hydroxyl group at C-4′; then an electron and a proton are added to C-2 to give **8**. This result indicated that **3** without an enone group caused by nucleophilic attack of C-5 at C-4′ of the carbonyl carbon. The yield of **8** was 11%, and the other products probably including compounds from decomposition of **8** were a complicated mixture, and not identified.



8

2.4. Biological activity

The biological activity of new compounds **4**, **5**, **6** and **8** were tested in two assays: lettuce seed germination, and elongation of the second leaf sheath of rice seedlings. The biological activity of **1** was tested as a control, and the half-maximal inhibition (IC_{50}) of **1** was about 3 μM in both assays. Compounds **4**, **5**, **6** and **8** were biologically inactive at concentrations from 0.1 to 100 μM in both assays. The affinity of **4** and **5** to the receptor would be lost since the side-chains have lost the conjugated double bond, and the conformations have changed. Compound **6** could not bind to the receptor since it is a dimer connected at C-4′ in the cyclohexenone ring which is important for binding to the receptor (Umezawa et al., 2010). No activity of **8** was to be expected since biological activity of **3** itself is very low (Hirai, 2010).

3. Conclusions

It has been determined that ABA methyl ester (**1**-**Me**) is electrochemically reduced to give unique products **4**-**Me** and **5**-**Me**. Phaseic acid (**3**) gave also a unique product **8** on cathodic reaction. Synthesis of these compounds by conventional methods seems to be difficult. Unique derivatives of ABA may be synthesized using the cathodic reaction.

4. Experimental

4.1. General methods

^1H and ^{13}C NMR, HMQC and HMBC spectra were measured with Bruker ARX500 (500 MHz for ^1H) and Avance 400 (400 MHz for ^1H) instruments using TMS as an internal standard. Mass spectra were measured with a JEOL JMS-600H mass spectrometer. UV and IR spectra were recorded with Shimadzu UV-2200AI and FTIR DR-8030 spectrometers, respectively. Optical rotations were measured with a JASCO DIP-1000 polarimeter. Column chromatography (CC) was carried out on silica gel (Wakogel C-200, Wako Pure Chemical Industries, Osaka, Japan) and ODS gel (AM 120-S50, YMC, Kyoto, Japan). Preparative HPLC was performed with an ODS column (YMC AQ-311, 6 × 100 mm) or a silica gel column (YMC Pack-SIL A-003, 4.6 × 250 mm).

4.2. Materials

(+)-ABA (**1**) was a gift from Dr. Y. Kamuro of BAL Enterprise Corp. (Ichinomiya, Japan). Its methyl ester (**1**-**Me**) was prepared by treatment of **1** with an Et_2O solution of diazomethane. Phaseic acid (**3**) was prepared by alkaline hydrolysis of 3-hydroxy-3-methylglutaryl ester of 8′-hydroxy-ABA which was isolated from immature seeds of *Robinia pseudacacia* L grown in the north campus of Kyoto University (Hirai et al., 1978). Seeds of lettuce (*Lactuca sativa* L. cv. Cisco) were purchased from a gardening shop in Kyoto City, and seeds of rice (*Oryza sativa* L. cv. Nihonbare) were supplied by the Experimental Farm, Graduate School of Agriculture, Kyoto University.

4.3. Cyclic voltammetry and electrolysis

Cyclic voltammetric experiments and electrolysis were carried out using an HSV-100 instrument (Hokuto Denko, Tokyo, Japan). H-type cells of two sizes with sintered glass separators were used. The sizes of cathode and anode compartments were 12 ml and 3 ml, respectively, and 20 ml and 5 ml, respectively. Electrode potentials were measured against Ag/AgCl reference electrode immersed in a satd.d KCl solution separated by a salt bridge (agar containing KCl) from the reaction solution A glassy carbon rod was used as a working electrode in cyclic voltammetric experiments. A mercury pool was used as a working electrode in bulk electrolysis. The counter electrode was a platinum wire. Electrolysis was carried out at −2.0 V for 4.5 h under argon atmosphere. The buffer solutions used were 50 mM citric acid buffer of pH 3, 50 mM phosphate buffer of pH 7, and 50 mM borate buffer of pH 10. Compounds **1** and **3** dissolved in EtOH (0.1 or 0.2 ml) were added to 14.9 or 24.8 ml of the buffer solutions, respectively, and **1**-**Me** dissolved in CH_3CN (3 or 5 ml) of CH_3CN was added to 12 or 20 ml of the buffer solutions, respectively. The total volume of the buffer solutions used for bulk electrolysis was 15 or 25 ml, and the concentration of **1**, **1**-**Me** and **3** was 5 mM. As the supporting electrolyte 0.1 M $(C_2H_5)_4NClO_4$ (Nacalai Tesque, Kyoto, Japan) was used. Products in the reaction solutions of cathode were analyzed by HPLC. For chase of Terem and Utley's experiment, the electrolysis conditions were as reported by them (Terem and Utley, 1979).

4.4. HPLC analysis

For HPLC analyses of the reaction solutions in the cathode compartment, an ODS column (YMC AQ-311, 6 × 100 mm) was used at

a flow rate 1.0 ml/min. The reaction solution after electrolysis of **1**-**Me** was eluted with a mixture of MeOH–H_2O–HOAc (25:75:0.1–40:60:0.1; a linear gradient by 50 min) and detection at 235 nm. The t_R values were 35.8 min for **5**-**Me**, 50.4 min for **4**-**Me**, and 51.6 min for **1**-**Me**. The reaction solution after electrolysis of **1** was analyzed using two conditions; elution with a mixture of MeOH–H_2O–HOAc (25:75:0.1–40:60:0.1; a linear gradient for 40 min) and detection at 235 nm gave **5** at 17.5 min, and elution with a mixture of MeOH–H_2O–HOAc (40:60:0.1–50:50:0.1; a linear gradient by 20 min) and detection at 254 nm gave **6** and **7** at 10.9 min and 16.2 min, respectively. The reaction solution after electrolysis of **3** was analyzed with a mixture of MeOH–H_2O–HOAc (5:95:0.1) and detection at 220 nm, and **8** was eluted at 17.4 min. The amounts of compounds were calculated by the calibration curves between peak area and weight.

4.5. Electrolysis of ABA methyl ester (**1**-**Me**), and isolation of compound **5**-**Me**

Compound **1**-**Me** (35 mg) in 25 ml of the pH 7 buffer solution containing CH_3CN–H_2O (1:4, v/v) was electrolyzed at −2.0 V for 4.5 h. The electrolysis solution (20 ml) in the cathode compartment was diluted with H_2O to 30 ml, and extracted with (4 × 20 ml) (EtOAc). The combined organic solubles were washed with H_2O, dried over Na_2SO_4, and concentrated to give a crude product (27 mg). The latter was purified on an ODS gel (YMC AM 120-S50, 6 g) column with a mixture of MeOH–H_2O (30:70–40:60) as eluant and the eluate was collected in fractions of 13 ml. Fraction nos. 38–43 (total 78 ml) were combined, diluted with H_2O to 160 ml, and extracted with EtOAc (100 ml × 4). The organic layer was washed with H_2O, dried over (Na_2SO_4), and concentrated to give **5**-**Me** (15 mg).

4.5.1. (−)-(E)-methyl 4-((1S,6R,7R)-1-hydroxy-2,2,6-trimethyl-4-oxobicyclo[4.1.0]heptan-7-yl)-3-methylbut-3-enoate (**5**-**Me**)

White wax-like material, 54% yield for the cathode solution; $[\alpha]_D^{28}$ −44.0 (*c* 0.40, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$):δ 1.13 (3H, *s*, H-9′), 1.15 (3H, *s*, H-7′), 1.19 (3H, *s*, H-8′), 1.67 (1H, *d*, *J* = 7.3 Hz, H-5), 1.76 (3H, *d*, *J* = 1.0 Hz, H-6), 2.04 (1H, *s*, 1′-OH), 2.22 (2H, *s*, H-5′), 2.60 (1H, *d*, *J* = 19.6 Hz, H-3′$_{pro\text{-}S}$),2.68 (1H, *d*, *J* = 19.6 Hz, H-3′$_{pro\text{-}R}$),3.08 (2H, *s*, H-2), 3.69 (3H, *s*, 1-*O*-Me), 5.20 (1H, *dd*, *J* = 7.3 and 1.0 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7 (C-7′), 17.4 (C-6), 25.0 (C-2′), 25.2 (C-8′), 25.9 (C-5), 26.1 (C-9′), 36.3 (C-6′), 44.7 (C-2), 46.9 (C-3′), 51.9 (1-*O*-Me), 52.0 (C-5′), 66.5 (C-1′), 122.5 (C-4), 132.6 (C-3), 172.4 (C-1), 209.5 (C-4′); UV λ nm (log ε): 206 (3.77), $MeOH_{max}$; IR ν_{max} ($CHCl_3$) cm^{-1}: 3736, 2958, 2932, 2363, 2342, 1734, 1722, 1542, 1458, 1437; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 280 $[M]^+$ (10), 278 (3), 262 $[M\text{-}H_2O]^+$ (5), 252 $[M\text{-}CO]^+$ (8), 221 $[M\text{-}CO_2Me]^+$ (15), 207 (12), 181 (25), 168 (12), 154 (100), 149 (25), 137 (13), 127 (24), 123 (34), 121 (47), 112 (16), 109 (36), 95 (35), 85 (33); HR-EIMS: *m*/*z* 280.1675 $[M]^+$ (calcd. for $C_{16}H_{24}O_4$ 280.1674).

4.6. Electrolysis of ABA methyl ester (**1**-**Me**), and isolation of compound **4**-**Me**

Compound **1**-**Me** (30 mg) in 50 mM borate buffer solution of pH 10 (25 ml) containing CH_3CN—H_2O (1:4, v/v) was electrolyzed at −2.0 V for 4.5 h. The electrolysis solution (17 ml) in the cathode compartment was diluted with H_2O to 30 ml, and extracted with EtOAc (20 ml × 4). The combined organic solubles were washed with H_2O, dried over (Na_2SO_4), and concentrated to give a crude product (31 mg). The product was purified on an ODS gel (YMC AM 120-S50, 6 g) with a mixture of MeOH–H_2O (30:70–40:60) as dust to give colorless oil (22 mg), which was further purified This oil was chromatographed on a silica gel column (Wakogel C-200, 15 g) with a mixture of toluene–EtOAc (90:10–80:20) to give **4**-**Me** (12 mg).

4.6.1. (S,E)-methyl 3-methyl-5-(1,3,3-trimethyl-2,5-dioxocyclohexyl)pent-3-enoate (**4**-**Me**)

Colorless oil, yield 40%; $[\alpha]_D^{28}$ +91.3 (*c* 0.40, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.14 (3H, *s*, H-9′), 1.19 (3H, *s*, H-7′), 1.20 (3H, *s*, H-8′), 1.67 (3H, *s*, H-6), 2.07 (1H, *dd*, *J* = 14.0 and 8.9 Hz, H-5), 2.46 (1H, *d*, *J* = 18.1 Hz, H-3′$_{pro\text{-}S}$), 2.55 (1H, *dd*, *J* = 14.0 and 7.3 Hz, H-5), 2.58 (1H, *d*, *J* = 18.3 Hz, H-5′$_{pro\text{-}R}$), 2.61 (1H, *d*, *J* = 18.3 Hz, H-5′$_{pro\text{-}S}$), 2.86 (1H, *d*, *J* = 18.1 Hz, H-3′$_{pro\text{-}R}$), 2.98 (2H, *s*, H-2), 3.65 (3H, *s*, 1-*O*-Me), 5.13 (1H, *dd*, *J* = 7.3 and 8.9 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.7 (C-6), 25.9 (C-9′), 26.2 (C-7′), 26.5 (C-8′), 37.6 (C-5), 43.5 (C-6′), 45.0 (C-2), 47.6 (C-2′), 47.8 (C-3′), 50.4 (C-5′), 51.8 (1-*O*-Me), 123.9 (C-4), 132.8 (C-3), 171.9 (C-1), 208.8 (C-4′), 215.8 (C-1′); UV λ nm (log ε): 204 (3.72), $MeOH_{max}$; IR ν_{max} ($CHCl_3$) cm^{-1}: 2980, 1736, 1712, 1192, 1005; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 280 $[M]^+$ (13), 262 $[M\text{-}H_2O]^+$ (4), 249 $[M\text{-}OCH_3]^+$ (8), 220 (8), 207 (8), 181 (17), 154 (100), 127 (29), 109 (30), 95 (37), 85 (38); HR-EIMS: *m*/*z* 280.1677$[M]^+$ (calcd. for $C_{16}H_{24}O_4$ 280.1674). The twist-boat and half-chair conformers of **4**-**Me** were fully optimized with density functional theory, using the Becke three parameter hybrid functional (B3LYP) method and the 6-31G(d) basis set in Gaussian 09 (Gaussian 09, 2009), followed by a calculation of the harmonic vibrational frequencies at 298 K at the same level. The single point energies were calculated with B3LYP/6-311++G(2df,2p) level of theory. The zero-point energies were scaled by 0.9804.

4.7. Electrolysis of ABA methyl ester (**1**-**Me**) in a D_2O buffer, and isolation of compound **5**-**Me**-d_2

A buffer solution (12 ml) of pH 7 was prepared using D_2O, and **1**-**Me** (20 mg) dissolved in CH_3CN (3 ml) solution was added to the buffer solution Electrolysis was carried out at −2.0 V for 4.5 h. The electrolysis solution (12 ml) in the cathode compartment was extracted with 10 ml of EtOAc (10 ml × 4). The organic solubles were combined, washed with H_2O, dried (Na_2SO_4), and concentrated to give colorless oil (23 mg). This oil was chromatographed on ODS gel (YMC AM 120-S50, 6 g) eluted with a mixture of MeOH–H_2O (30:70–50:50). The eluate was collected in fractions of 13 ml, and fractions no. 24–38 were concentrated to give crude a crude product (10 mg). The crude product was applied to a ODS gel (YMC AM 120-S50, 6 g) with a mixture of MeOH–H_2O (30:70–40:60) as eluant and the eluate was collected in fractions of 10 ml. Fraction nos. 33–47 (total 150 ml) were combined and concentrated to 100 ml, and extracted with EtOAc (50 ml × 4). The organic layers were combined, washed with H_2O, dried (Na_2SO_4), and concentrated to give **5**-**Me**-d_2 (8.4 mg).

4.7.1. Compound **5**-**Me**-d_2

White wax-like material; ^{1}H NMR (500 MHz, $CDCl_3$):δ 1.13 (3H, *s*, H-9′), 1.15 (3H, *s*, H-7′), 1.18 (3H, *s*, H-8′), 1.68 (1H, *d*, *J* = 7.2 Hz, H-5), 1.76 (3H, *s*, H-6), 1.95 (1H, *br s*, 1′-OH), 2.21 (2H, *s*, H-5′), 2.57 (0.14H, *br s*, H-3′$_{pro\text{-}S}$), 2.60 (0.27H, *d*, *J* = 19.7 Hz, H-3′$_{pro\text{-}S}$), 2.66 (0.59H, *br s*, H-3′$_{pro\text{-}R}$), 2.68 (0.27H, *d*, *J* = 19.7 Hz, H-3′$_{pro\text{-}R}$), 3.07 (0.88H, *s*, H-2), 3.08 (0.23H, *s*, H-2), 3.69 (3H, *s*, 1-*O*-Me), 5.20 (1H, *d*, *J* = 7.2 Hz, H-4); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 282 $[Md_2]^+$ (8), 281 $[Md_1]^+$ (4), 280 $[Md_0]^+$ (2), 264 $[Md_2\text{-}18]^+$ (4), 254 (8), 223 (16), 182 (25), 155 (100), 122 (54); the deuterium content at C-2 and -3′ was 71.0%.

4.8. Mulliken charge and spin density of ABA methyl ester (**1**-**Me**)

A half-chair conformation of **1**-**Me** with the side-chain axial was used as the primary structure, and geometry optimization was

carried out by B3LYP/6-31G(d) method with electric charge −1, spin multiplicity 2, and open shell. The method and basis set for calculation of Mulliken charge and spin density was B3LYP/6-31 + G(d,p) method with electric charge −1, spin multiplicity 2, and open shell (Gaussian 98, 1998).

4.9. Electrolysis of ABA (**1**), and isolation of compound **6**

ABA (**1**, 21 mg) in 15 ml of the buffer solution of pH 7 was electrolyzed at −2.0 V for 4.5 h. The electrolysis solution (13 ml) in the cathode compartment was acidified with 1 M HCl to pH 2 and extracted with EtOAc (10 ml × 4). The combined organic solubles were washed with H_2O, dried (Na_2SO_4), and concentrated to give colorless oil (21 mg). The latter applied to a silica gel (Wakogel C-200, 14 g) column eluted with a mixture of toluene–EtOAc–HCO_2H (70:30:0.1–40:60:0.1). The compounds eluted with 50–60% EtOAc in toluene were combined, and purified by preparative HPLC using an ODS column (YMC AQ-311, 6 × 100 mm) with MeOH–H_2O–HCO_2H (50:50:0.1–65:35:0.1, 12 min linear gradient). A compound eluted at 5.0 min was collected, and concentrated to give **6** (1.0 mg).

4.9.1. 5-[10-(4-carboxy-3-methyl-buta-1,3-dienyl)-3,10-dihydroxy-2,4,4,9,11,11-hexamethyl-13-oxa-dispiro[5.0.5.1]trideca-1,8-dien-3-yl]-3-methyl-penta-2,4-dienoic acid (**6**)

Colorless oil, yield 4.9%; $[\alpha]_D^{22}$ +366° (*c* 0.10, MeOH); ^{1}HNMR (500 MHz, CD_3OD, 325 K): δ 0.87 (3H, *s*, H-9′ or-8′), 1.14 (3H, *s*, H-8′or -9′), 1.33 (2H, *m*, H-5′), 1.72 (3H, *s*, H-7′), 194 (3H, *s*, H-6), 5.68 (2H, *s*, H-2 and H-3′), 6.02 (1H, *m*, H-5), 7.76 (1H, *d*, *J* = 16.1 Hz, H-4); UV λ nm (log ε): 262 (4.43), $MeOH_{max}$. Compound **6** was treated with diazomethane to give a dimethyl ester **6-Me**. Spectroscopic data of **6-Me**: $[\alpha]_D^{29}$ +437° (*c* 0.11, MeOH); ^{1}HNMR (500 MHz, CD_3OD, 325 K): δ 0.86 (3H, *s*, H-9′ or H-8′), 1.16 (3H, *s*, H-8′ or H-9′), 1.36 (1H, *d*, *J* = 14.8 Hz, H-5′), 1.58 (1H, *d*, *J* = 14.8 Hz, H-5′), 1.74 (3H, *s*, H-7′), 1.95 (3H, *s*, H-6), 3.67 (3H, *s*, 1-*O*-Me), 5.68 (1H, *s*, H-2), 5.69 (1H, *s*, H-3′), 6.08 (1H, *d*, *J* = 16.1 Hz, H-5), 7.64 (1H, *d*, *J* = 16.1 Hz, H-4); ^{13}CNMR (75 MHz, $CDCl_3$): δ 18.3 (C-7′), 21.0 (C-6), 24.2 (C-8′ or C-9′), 25.5 (C-9′ or C-8′), 37.7 (C-6′), 41.4 (C-5′), 51.1 (1-*O*-Me), 74.4 (C-4′), 79.3 (C-1′), 117.5 (C-2), 124.9 (C-3′), 127.1 (C-4), 138.7 (C-5), 140.5 (C-2′), 149.9 (C-3), 166.3 (C-1); UV λ nm (log ε): 267 (4.30), $MeOH_{max}$; IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 2953, 1713, 1707, 1606, 1245, 1221, 1167; EIMS (probe) 70 eV, *m/z* (rel. int.): 540 $[M]^+$ (2), 522 $[M-H_2O]^+$ (26), 504 $[M-2H_2O]^+$ (38), 490 (18), 486 $[M-3H_2O]^+$ (35), 472 (22), 439 (22), 262 (100), 247 (33), 125 (75); HR–EIMS: *m/z* 540.3097 $[M]^+$ (calcd for $C_{32}H_{44}O_7$ 540.3087).

4.10. Electrolysis of ABA (**1**), and isolation of 1′-deoxy-ABA (**7**)

ABA (**1**, 23 mg) in 15 ml of the pH 10 buffer solution was electrolyzed at −2.0 V for 4.5 h. The cathodic solution (13 ml) was acidified with 1 M HCl to pH 2 and extracted with of EtOAc (10 ml × 4). The combined organic solubles were washed with H_2O, dried (Na_2SO_4), and concentrated to give colorless oil (21 mg). The latter subjected to silica gel (Wakogel C-200, 14 g) CC with toluene–EtOAc–HCO_2H (20:80:0.05–60:40:0.05). The substances eluted with 20–30% EtOAc in toluene were purified by preparative HPLC using an ODS column (YMC AQ-311, 6 × 100 mm) with a mixture of MeOH–H_2O–HCO_2H (45:55:0.01–50:50:0.01, a linear gradient by 12 min). The substances eluted at 11.7 min were further purified by preparative HPLC using an ODS column (YMC AQ-311, 6 × 100 mm) with a mixture of MeOH–H_2O–HCO_2H (45:55:0.01). The compound eluted at 13.0 min was collected, and concentrated to give **7** (1.3 mg, 5.7% yield). Compound **7** did not show any significant optical rotation.

4.11. Electrolysis of ABA (**1**) in a D_2O buffer, and isolation of compounds **6** and **7-d_2**

A buffer solution (15 ml) of pH 10 was prepared using D_2O, and ABA (21 mg) was electrolyzed in the buffer at −2.0 V for 4.5 h. The electrolysis solution (13 ml) in the cathode compartment was acidified with 1 M HCl to pH 2 and extracted with EtOAc (10 ml × 4). The combined organic solubles were washed with H_2O, dried (Na_2SO_4), and concentrated to give crude products (19 mg). The products were chromatographed on silica gel (Wakogel C-200, 30 g) column eluted with a mixture of toluene–EtOAc–HOAc (90:10:0.1–40:60:0.1). The colorless oil (2.0 mg) eluted with 20% and 30% EtOAc was purified by preparative HPLC using an ODS column (YMC AQ-311, 6 × 100 mm) with MeOH–H_2O–HOAc (45:55:0.1) and detection at 254 nm. The substances eluted at 12.9 min was collected, and concentrated to give colorless oil (1.2 mg). The latter was purified by preparative HPLC using a silica gel column (YMC PackSIL A-003) with a mixture of $CHCl_3$–HOAc (100:0.1) and detection at 254 nm. A material eluted at13.7 min was collected, and concentrated to give **7-d_2** (0.2 mg). The substance eluted from the silica gel column with 50% and 60% EtOAc was concentrated to give **6** (3.0 mg).

4.11.1. 1′-Deoxy-ABA-d_2 (**7-d_2**)

Colorless oil; ^{1}HNMR (500 MHz, $CDCl_3$): δ 0.99 (3H, *s*, H-9′), 1.05 (3H, *s*, H-8′), 1.95 (3H, *s*, H-7′), 1.99 (3H, *s*, H-6), 2.08 (1H, *d*, *J* = 16.9 Hz, H-5′), 2.47 (1H, *d*, *J* = 16.8 Hz, H-5′), 2.81 (0.35H, *d*, *J* = 9.6 Hz, H-1′), 5.74 (1H, *s*, H-3′), 5.91 (0.11H, *s*, H-2), 6.00 (1H, *d*, *J* = 15.7 Hz, H-5), 7.66 (1H, *d*, *J* = 15.7 Hz, H-4). Compound **7-d_2** was treated with diazomethane to give a monomethyl ester **7-Me-d_2**. EIMS (probe) 70 eV, *m/z* (rel. int.): 264 $[Md_2]^+$ (0.3), 263 $[Md_1]^+$ (0.2), 262 $[Md_0]^+$ (0.1), 125 (100); the total deuterium content at C-2 and -1′ was 55.8%.

4.12. Electrolysis of phaseic acid (**3**), and isolation of compound **8**

Phaseic acid (**3**, 9 mg) was electrolyzed in 15 ml of a pH 3 buffer solution at −2.0 V for 4.5 h. The total electric charge was 9.9 C corresponding to 170% of electric charge to reduce all **7**. The cathodic solution (12 ml) was recovered, diluted with H_2O, and partitioned with EtOAc (20 ml × 4). The combined organic solubles were washed with H_2O, and dried (Na_2SO_4). After filtration the organic layer was concentrated to give a solid (5.8 mg). The solid was applied to silica gel (Wakogel C-200, 1.2 g), and eluted with mixtures of toluene and EtOAc. The material eluted with 80% EtOAc in toluene was injected to an HPLC column (YMC AQ-311, 6 × 100 mm), and eluted with a mixture of MeOH–H_2O–HOAc (5:95:0.1) at a flow rate of 1.0 ml/min and detected at UV 220 nm. A material eluted at 14.0 min was collected, and concentrated to give **8** (1 mg).

4.12.1. (E)-4-(3a,5-dihydroxy-3,6a-dimethylhexahydro-2H-3,5-methanocyclopenta[b]furan-4-yl)-3-methylbut-3-enoic acid (**8**)

Colorless solid; $[\alpha]_D^{28}$ +13° (*c* 0.1, MeOH);^{1}HNMR (500 MHz, CD_3OD): δ 1.09 (3H, *s*, H-9), 1.30 (3H, *s*, H-7′), 1.71 (1H, *d*, *J* = 12.4 Hz, H-5′), 1.78 (1H, *d*, *J* = 11.9 Hz, H-3′), 1.79 (3H, *s*, H-6), 1.82 (1H, *d*, *J* = 12.4 Hz, H-5′), 2.04 (1H, *d*, *J* = 11.9 Hz, H-3′), 2.94 (1H, *d*, *J* = 9.6 Hz, H-5), 3.09 (2H, *s*, H-2), 3.65 (1H, *d*, *J* = 7.6 Hz, H-8′$_{pro\text{-}S}$), 3.67 (1H, *d*, *J* = 7.6 Hz, H-8′$_{pro\text{-}R}$), 5.40 (1H, *d*, *J* = 9.6 Hz, H-4); ^{13}CNMR (125 MHz, CD3OD): δ 15.6 (C-6 or C-9′), 17.2 (C-7′), 21.5 (C-9′ or C-6), 45.5 (C-2), 46.5 (C-6′), 49.0 (C-3′), 50.8 (C-5′), 53.1 (C-5), 74.1 (C-4′), 77.0 (C-8′), 87.5 (C-1′), 87.7 (C-2′), 121.8 (C-4), 135.3 (C-3), 175.6 (C-1). Compound **8** (1 mg) was treated with diazomethane to give **8-Me** (1 mg). Spectral data of **8-Me**. $[\alpha]_D^{26}$ +14° (*c* 0.1, MeOH);^{1}HNMR (500 MHz, CD_3OD):δ 1.09 (3H, *s*, H-9′), 1.28 (3H, *s*, H-7′), 1.70 (1H, *d*, *J* = 11.9 Hz, H5′), 1.77 (1H, *d*,

J = 11.9 Hz, H-5′), 1.78 (3H, *s*, H-6), 1,79 (1H, *d*, J = 13.1 Hz, H-3′), 2.03 (1H, *d*, J = 13.1 Hz, H-3′), 2.94 (1H, *d*, J = 9.2 Hz, H-5), 3.13 (2H, *s*, H-2), 3.65 (2H, *s*, H-8′), 3.68 (3H, *s*, 1-*O*-Me), 5.41 (1H, *d*, J = 9.2 Hz, H-4); EIMS (probe) 70 eV, m/z (rel. int.): 296 $[M]^+$ (3), 278 $[M\text{-}H_2O]^+$ (100), 237 (22), 223 (25), 205 (45), 181 (20), 170 (41), 165 (24), 139 (18), 95 (25), 83 (21); HR–EIMS: m/z 296.1589 $[M]^+$ (calcd. for $C_{16}H_{24}O_5$ 296.1624).

4.13. Bioassay

For lettuce seed germination assays, 0.1, 0.3, 1.0, 3.0, 10, 30 and 100 μM test solutions of compounds **1**, **4**, **5**, **6** and **8** were prepared by addition of distilled H_2O (3 ml) to dried compounds in Petri dishes. Two sheets of filter paper (No. 2 filter, 5.5 cm in diameter, Toyo Roshi Kasisha Ltd., Tokyo, Japan) were soaked in the test solution in each Petri dish. Fifty seeds of lettuce were placed on the filter paper, and allowed to germinate under illumination at 25 °C. After 48 h, the inhibition ratio was calculated. The inhibition ratio was defined as $[(A-B)/A] \times 100$, where A = the number of seeds that germinated when water was used, and B = the number of seeds that germinated when a test compound was used. The number of A was 49–50.

For rice elongation assay, test solutions of compounds **1**, **4**, **5**, **6** and **8** were prepared in the same manner as that described above but using glass tubes. Seeds of rice were soaked in EtOH for 5 min, sterilized with 1% antiformin ($NaClO_4$) for 1 h, and washed with running tap H_2O for 1 h. The sterilized seeds were allowed to germinate in H_2O for 2 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 7 days, and the inhibition ratio was calculated. The inhibition ratio was defined as $[(A-B)/A] \times 100$, where A = the mean length 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. The value of A was 23.0–23.8 mm.

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