JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2005; **40**: 1035–1043

JMS

Mechanism of the formation of a dehydrated ion by an unusual loss of oxygen at the 4′-carbonyl group of abscisic acid methyl ester in electron ionization mass spectrometry

Masahiro Inomata,[1] Nobuhiro Hirai,[2]* Naohito Takeda[3] and Hajime Ohigashi[1]

[1] Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[2] International Innovation Center, Kyoto University, Kyoto 606-8501, Japan
[3] Faculty of Pharmacy, Meijo University, Nagoya 468-8503, Japan

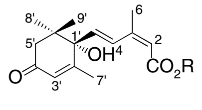
Received 18 February 2005; Accepted 13 April 2005

Methyl ester of abscisic acid (ABA), a plant hormone, gives a dehydrated ion at *m/z* 260 in electron ionization mass spectrometry (EI–MS). This dehydrated ion had been considered to be derived only from the elimination of the tertiary hydroxyl group at C-1′. We found that 34% of the dehydrated ion was formed by elimination of the oxygen atom at the 4′-carbonyl group, and the remaining 66% by elimination of the 1′-hydroxyl group. This unusual elimination of the carbonyl oxygen was shown with [4′-^{18}O]ABA methyl ester. Involvement of the 4′-carbonyl oxygen in dehydration was observed in methyl ester of phaseic acid (PA), a natural metabolite of ABA, but not in 1′-deoxy-ABA methyl ester or isophorone. This suggested that the 1′-hydroxyl group was necessary for the elimination of the 4′-carbonyl oxygen. ABA methyl esters labeled with stable isotopes showed that hydrogen atoms at the 1′-hydroxyl group and at C-4 or -5 or -3′ or - 5′ or -7′ were eliminated with the 4′-carbonyl oxygen. These results allow us to propose a formation mechanism of the dehydrated ion derived from the elimination of 4′-carbonyl oxygen and hydrogen atoms at C-4 and 1′-oxygen in ABA methyl ester as follows: first, ionization at the 1′-hydroxyl group occurs to give an ion radical, and the proton at the 1′-oxygen migrates to the 4′-carbonyl oxygen after the bond fission between C-1′–C-6′; second, migration of the proton at C-4 to the 1′-oxygen is followed by migration of the protons at C-5 and C-7′ to C-4 and C-5, respectively; finally, the proton at the 1′-oxygen migrates to the 4′-hydroxyl group, and H_2O at C-4′ is eliminated to give the dehydrated ion. Our findings point out that a dehydrated ion is not always derived from the elimination of a hydroxyl group. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: electron ionization mass spectrometry; dehydrated ion; abscisic acid; phaseic acid

INTRODUCTION

A plant hormone, abscisic acid (ABA, **1**) is a sesquiterpenoid which induces tolerance to various forms of abiotic stress including dehydration and low temperature, and regulates seed dormancy and germination.[1] ABA is produced not only by higher plants but also by fungi,[2] although the physiological function of ABA in fungi is unknown.



1: R = H
1-Me: R = Me

The EI mass spectrum of ABA methyl ester gives fragment ions at m/z 260 $[M - H_2O]^+$, 246 $[M - MeOH]^+$, 222, 205, 190, 162, 134, 125 and 112 in addition to a molecular ion at m/z 278 (Fig. 1a). Gray *et al.* proposed the structures and formation mechanism of these fragment ions on the basis of an analysis of ABA methyl esters labeled with stable isotopes.[3] The fragment ions at m/z 190 and 125 with high intensities are formed by loss of methanol at C-1 following elimination of isobutene consisting of carbon atoms at C-6′, -8′ and -9′ from the molecular ion, and by cleavage of the intact side chain at C-5–C-1′, respectively. They suggested that the dehydrated ion at m/z 260 was formed by elimination of the tertiary hydroxyl group at C-1′, but a hydrogen atom eliminated with the hydroxyl group had not been identified. Hirai *et al.* revealed that about 65% of the dehydrated ion was formed by elimination of the 1′-hydroxyl group and 4- or 5-hydrogen atoms bonded to a double bond, and proposed the structure of the dehydrated ion.[4] However, the eliminating group giving the residual 35% of dehydrated ion was still

---

*Correspondence to: Nobuhiro Hirai, International Innovation Center, Kyoto University, Kyoto 606-8501, Japan.
E-mail: hirai@kais.kyoto-u.ac.jp

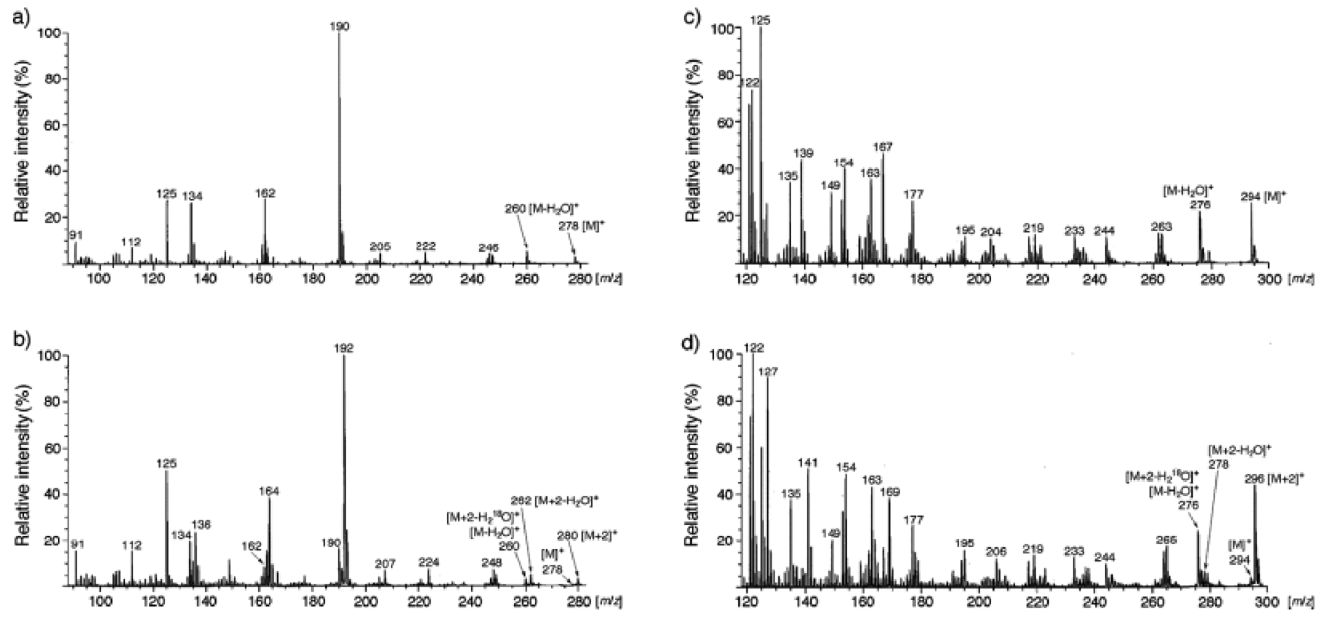


Figure 1. EI mass spectra of methyl esters of ABA (a), [4′-^{18}O]ABA (b), PA (c) and [4′-^{18}O]PA (d). Relative intensities are not corrected by natural ^{13}C-, ^{2}H- and ^{17}O-isotopic abundance.

unknown. Recently, we found that 35% of the dehydrated ion was formed by loss of the oxygen at the 4′-carbonyl group, in the course of labeling studies of ABA produced by a fungus, *Botrytis cinerea*, with $^{18}O_2$ and $H_2{}^{18}O$.[5] This paper describes the unusual involvement of the 4′-carbonyl oxygen atom in the formation of the dehydrated ion, and proposes a dehydration mechanism based on an analysis of compounds labeled with stable isotopes.

EXPERIMENTAL

General experimental procedures

^{1}H and ^{17}O NMR spectra were measured with a Bruker ARX500 instrument (500 MHz for ^{1}H and 68 MHz for ^{17}O). Chemical shifts were given with TMS (δ 0.00 ppm) as the internal standard for ^{1}H NMR analysis, and with H_2O (δ 0.00 ppm) as the external standard for ^{17}O NMR analysis. Direct EI mass spectra were measured with a JEOL JMS-600H mass spectrometer, the temperature of the direct probe being increased from 50 °C to 450 °C at a rate of 128 °C/min. The instrument was operated at a chamber temperature of 250 °C, an accelerating voltage of 3 kV, an ionization voltage of 70 eV, and an ionization current of 300 µA in the positive ion mode, the resolution being 1000 during the measurement. The GC/EI mass spectrum of compound **4** was recorded on the above mass spectrometer equipped with a Hewlett Packard HP6890 instrument, using an HP-5 column (30 m length ×0.32 mm i.d., 5% diphenyl-95% dimethylpolysiloxane, film thickness 0.25 µm, Hewlett Packard Co., Wilmington, DE, USA), and 1.0 ml/min of He flow. The parameters of the mass spectrometer were the same as those for direct electron ionization mass spectrometry(EI–MS). The oven temperature for GC was programmed to increase from 50 °C to 100 °C at a rate of 5 °C/min. ABA and phaseic acid (PA) methyl esters and their labeled compounds (except for [4′-^{17}O]ABA methyl ester) were measured in profile mode, and the relative intensities of the ions were calculated from peak areas. The mass spectra of [4′-^{17}O]ABA methyl ester, and compounds **3** and **4** were given in the form of a bar graph. The content of deuterium atom of [1′-*O*-D,4′-^{18}O]ABA methyl ester, and ^{18}O contents of labeled ABA methyl esters and PA methyl ester were calculated after correction by natural ^{13}C-, ^{2}H- and ^{17}O-isotopic abundance. Percentages of dehydrated ions derived from loss of 1′-OH and 4′-O in EI–MS of ABA and PA methyl esters, and of hydrogen atoms involved in dehydration in EI–MS of ABA methyl esters were shown as the mean value ± SD of three scans.

Materials

(±)-ABA was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI, USA. Isophorone was purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. $H_2{}^{18}O$ (more than 95 atom% ^{18}O) was purchased from Nippon Sanso Co., Kanagawa, Japan. $H_2{}^{17}O$ (48 atom% ^{17}O), D_2O and CD_3OD were purchased from Isotec Inc., Miamisburg, OH, USA. Methyl esters of PA, [4′-^{18}O]PA,[6] 1′-deoxy-ABA,[7] [4-D]ABA,[4] [5-D]ABA[4] and [3′,5′,5′,7′,7′,7′-D_6]ABA[4] which had been prepared in our laboratory were used.

[4′-^{17}O] and [4′-^{18}O]ABA methyl ester

ABA (10 mg) was dissolved in 2 ml of MeOH, and an ether solution of CH_2N_2 was added. After standing for 1 h at room temperature, the solution was concentrated to give methyl ester of ABA (10 mg). ^{1}H NMR ($CDCl_3$) : δ 1.02 (3H, s, H-9′), 1.11 (3H, s, H-8′), 1.93 (3H, d, $J = 1.2$ Hz, H-7′), 2.01 (3H, d, $J = 1.0$ Hz, H-6), 2.30 (1H, d, $J = 17.1$ Hz, H-5′$_{\text{pro}R}$), 2.48 (1H, d, $J = 17.1$ Hz, H-5′$_{\text{pro}S}$), 3.71 (3H, s, 1-OCH_3), 5.76 (1H, s, H-2), 5.95 (1H, s, H-3′), 6.16 (1H, d, $J = 16.1$ Hz, H-5), 7.87 (1H, d, $J = 16.1$ Hz, H-4). EI–MS (probe) 70 eV: m/z (rel. int.): 278 $[M]^+$ (3), 260 (6), 246 (4), 222 (6), 205 (6), 190 (100), 162 (29), 134 (26), 125 (28), 112 (6), 91 (9). ABA methyl ester (10 mg) was dissolved in a mixture of 50 µl of acetic anhydride and

200 μl of $H_2{}^{17}O$, and the solution was stirred at 45–50 °C for 15 h. After the addition of 2 ml of toluene to the solution, the mixture was concentrated to give [4′-^{17}O]ABA methyl ester (10 mg). ^{17}O NMR ($CDCl_3$) : δ 541.9 (O-4′). EI–MS (probe) 70 eV: m/z (rel. int.): 280 $[M + 2]^+$ (1), 279 $[M + 1]^+$ (3), 278 $[M]^+$ (2), 262 (1), 261 (4), 260 (7), 247 (7), 223 (5), 206 (5), 205 (6), 191 (100), 190 (87), 163 (26), 162 (29), 135 (21), 134 (28), 125 (49), 112 (13), 91 (13); the ^{17}O content was calculated from relative intensities of the molecular ions and found to be 48%. In the same manner as described for the preparation of [4′-^{17}O] ABA methyl ester, ABA methyl ester (9 mg) was reacted with 45 μl of acetic anhydride and 180 μl of $H_2{}^{18}O$, and the reaction mixture was concentrated to give [4′-^{18}O] ABA methyl ester (9 mg). EI–MS (probe) 70 eV: m/z (rel. int.): 280 $[M + 2]^+$ (4), 278 $[M]^+$ (0.3), 262 (6), 260 (4), 248 (7), 224 (8), 207 (7), 192 (100), 190 (17), 164 (38), 162 (8), 136 (23), 134 (20), 125 (50), 112 (16), 91 (16).

EI mass spectra of methyl esters of PA and [4′-^{18}O] PA, and of compounds 3 and 4

PA methyl ester: EI–MS (probe) 70 eV: m/z (rel. int.): 294 $[M]^+$ (24), 276 (21), 263 (12), 177 (27), 167 (47), 163 (36), 154 (40), 139 (44), 135 (34), 125 (100), 122 (78), 121 (68). [4′-^{18}O]PA methyl ester: EI–MS (probe) 70 eV: m/z (rel. int.): 296 $[M + 2]^+$ (46), 294 $[M]^+$ (3), 278 (7), 276 (26), 265 (18), 177 (28), 169 (40), 163 (43), 154 (48), 141 (52), 135 (38), 127 (91), 125 (60), 122 (100), 121 (74); the ^{18}O content was calculated from relative intensities of the molecular ions and found to be 94%. Compound **3**: EI–MS (probe) 70 eV: 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), 103 (13), 91 (25). Compound **4**: GC/EI–MS (t_R 7.3 min) 70 eV: m/z (rel. int.): 138 $[M]^+$ (28), 123 (3), 95 (4), 82 (100), 67 (2), 54 (9).

[1′-*O*-D,4′-^{18}O]ABA methyl ester

[4′-^{18}O] ABA methyl ester (5 μg) in a quartz capillary (for a direct probe of mass spectrometer) was dissolved in 3 μl of CD_3OD, and the solution was dried by heating. After this procedure had been repeated twice more, the sample was dissolved in a mixture of 3 μl of CD_3OD and 2 μl of D_2O. The solution was concentrated to about 1 μl by heating, and the capillary was immediately introduced into the ionization chamber of the mass spectrometer with the direct probe. To reduce back-exchange by H_2O, 1 μl of D_2O in the capillary was introduced twice to the ionization chamber before analysis of the sample. Substitution of the 1′-hydroxyl proton with a deuterium ion was confirmed by the disappearance of the 1′-hydroxyl proton signal at 2.18 ppm in an ^{1}H NMR spectrum of a $CDCl_3$ solution of [4′-^{18}O] ABA methyl ester (9 mg/0.7 ml) after addition of 20 μl of D_2O. EI–MS (probe) 70 eV: m/z (rel. int.): 281 $[M + 3]^+$ (3), 280 $[M + 2]^+$ (1), 279 $[M + 1]^+$ (0.5), 263 (3), 262 (4), 261 (1), 260 (3), 248 (6), 225 (6), 208 (4), 193 (88), 192 (100), 191 (11), 190 (11), 165 (24), 164 (22), 136 (20), 134 (16), 125 (49), 112 (16), 91 (11). The deuterium content at the 1′-hydroxyl group and ^{18}O content at C-4′ were calculated from relative intensities of the molecular ions and found to be 79% and 88%, respectively. Since [1′-*O*-D,4′-^{18}O]ABA methyl ester was labeled with deuterium and ^{18}O, calculation of the compositions of the dehydrated ions was difficult. The compositions of the dehydrated ions were calculated using those of methyl esters of [4′-^{18}O]ABA and [1′-*O*-D]ABA: this method was used to calculate compositions of dehydrated ions of [4-D,4′-^{18}O]ABA, [5-D,4′-^{18}O]ABA and [3′,5′,5′,7′,7′,7′-D$_6$,4′-^{18}O]ABA methyl esters. The percentage of $[M + 3 - DH\,^{18}O]^+$ ion in the dehydrated ion formed by loss of 4′-^{18}O was calculated using the following equation:

$$\frac{25\%\ (\text{percent }[M + 3 - DH\,^{18}O]^+\text{ ion at } m/z\ 260)}{\{6\%\ (\text{percent }[M + 3 - H_2\,^{18}O]^+\text{ ion at } m/z\ 261) + 25\%\ (\text{percent}[M + 3 - DH\,^{18}O]^+\text{ ion at } m/z\ 260)\}} \times 100\% = 81\%$$

[4-D,4′-^{18}O]ABA methyl ester

[4-D,4′-^{18}O] ABA methyl ester (2 mg) was prepared from [4-D] ABA methyl ester (2 mg) containing 8% and 92% deuterium at H-2 and H-4, respectively, with 15 μl of acetic anhydride and 45 μl of $H_2{}^{18}O$ by the same method as that described for the preparation of [4′-^{18}O]ABA methyl ester. ^{1}H NMR ($CDCl_3$) : δ 1.02 (3H, s, H-9′), 1.11 (3H, s, H-8′), 1.93 (3H, d, $J = 1.2$ Hz, H-7′), 2.01 (3H, d, $J = 0.9$ Hz, H-6), 2.30 (1H, d, $J = 17.1$ Hz, H-5′$_{\text{proR}}$), 2.48 (1H, d, $J = 17.1$ Hz, H-5′$_{\text{proS}}$), 3.71 (3H, s, 1-OCH_3), 5.76 (92% of 1H, s), 5.95 (1H, s, H-3′), 6.15 (1H, br.s, H-5), 7.88 (8% of 1H, d, $J = 16.0$ Hz). EI–MS (probe) 70 eV: m/z (rel. int.): 282 $[M + 4]^+$ (0.9), 281 $[M + 3]^+$ (4), 280 $[M + 2]^+$ (0.6), 279 $[M + 1]^+$ (0.6), 264 (0.6), 263 (2), 262 (4), 261 (2), 260 (2), 249 (6), 225 (8), 208 (4), 193 (100), 192 (72), 191 (12), 190 (8), 165 (20), 164 (23), 137 (18), 135 (15), 126 (33), 113 (8), 92 (6). The ^{18}O content was calculated from relative intensities of the molecular ions to be 88%. The deuterium contents at H-2 and H-4 were unchanged, as confirmed by the ^{1}H NMR spectrum. The percentages of $[M + 3 - DH\,^{18}O]^+$ and $[M + 3 - DHO]^+$ ions in the dehydrated ions formed by loss of 4′-^{18}O and 1′-OH, respectively, were calculated using the following equations:

$$\text{Percent }[M + 3 - DH\,^{18}O]^+\text{ ion in the dehydrated ion formed by loss of 4}'\text{-}^{18}O = \frac{9\%\ (\text{percent }[M + 3 - DH\,^{18}O]^+\text{ ion at } m/z\ 260)}{\{21\%\ (\text{percent }[M + 3 - H_2\,^{18}O]^+\text{ ion at } m/z\ 261) + 9\%\ (\text{percent }[M + 3 - DH\,^{18}O]^+\text{ ion at } m/z\ 260)\}} \times 100\% = 30\%$$

$$\text{Percent }[M + 3 - DHO]^+\text{ ion in the dehydrated ion formed by loss of 1}'\text{-OH} = \frac{45\%\ (\text{percent }[M + 3 - DHO]^+\text{ ion at } m/z\ 262)}{\{24\%\ (\text{percent }[M + 3 - H_2O]^+\text{ ion at } m/z\ 263) + 45\%\ (\text{percent }[M + 3 - DHO]^+\text{ ion at } m/z\ 262)\}} \times 100\% = 65\%$$

Preparation of [5-D,4′-^{18}O]ABA methyl ester

[5-D,4′-^{18}O]ABA methyl ester (2 mg) was prepared from [5-D]ABA methyl ester (2 mg) containing 95% deuterium at H-5 with 15 μl of acetic anhydride and 45 μl of $H_2{}^{18}O$ by the same method as that described for the preparation of [4′-^{18}O]ABA methyl ester. ^{1}H NMR ($CDCl_3$) : δ 1.02 (3H, s, H-9′), 1.11 (3H, s, H-8′), 1.93 (3H, d, $J = 1.1$ Hz, H-7′), 2.01 (3H, s, H-6), 2.30 (1H, d, $J = 17.1$ Hz, H-5′$_{\text{proR}}$), 2.48 (1H, d,

$J = 17.1$ Hz, H-5′$_{\mathrm{proS}}$), 3.71 (3H, s, 1-OCH$_3$), 5.76 (1H, s, H-2), 5.95 (1H, s, H-3′), 6.16 (5% of 1H, d, $J = 16.1$ Hz), 7.87 (1H, br.s, H-4). EI–MS (probe) 70 eV: m/z (rel. int.): 281 $[M+3]^+$ (3), 280 $[M+2]^+$ (0.3), 279 $[M+1]^+$ (0.7), 263 (3), 262 (1), 261 (3), 260 (0.7), 249 (4), 225 (6), 208 (4), 193 (100), 192 (20), 191 (36), 190 (6), 165 (24), 164 (13), 137 (18), 135 (18), 126 (38), 112 (10), 92 (7). The ^{18}O content was calculated from relative intensities of the molecular ions and found to be 82%, and the deuterium content at H-5 was unchanged. The percentages of $[M+3-DH^{18}O]^+$ and $[M+3-DHO]^+$ ions in the dehydrated ions formed by loss of 4′-^{18}O and 1′-OH, respectively, were calculated using the following equations:

$$\begin{aligned}&\text{Percent } [M+3-DH^{18}O]^+ \text{ ion in the dehydrated}\\&\qquad\text{ion formed by loss of } 4'\text{-}^{18}O\\&= \frac{5\%\ (\text{percent } [M+3-DH^{18}O]^+ \text{ ion at } m/z\ 260)}{\{30\%\ (\text{percent } [M+3-H_2{}^{18}O]^+ \text{ ion at } m/z\ 261) + 5\%\ (\text{percent } [M+3-DH^{18}O]^+ \text{ ion at } m/z\ 260)\}}\\&\quad\times 100\% = 14\%\end{aligned}$$

$$\begin{aligned}&\text{Percent } [M+3-DHO]^+ \text{ ion in the dehydrated}\\&\qquad\text{ion formed by loss of } 1'\text{-OH}\\&= \frac{9\%\ (\text{percent } [M+3-DHO]^+ \text{ ion at } m/z\ 262)}{\{55\%\ (\text{percent } [M+3-H_2O]^+ \text{ ion at } m/z\ 263) + 9\%\ (\text{percent } [M+3-DHO]^+ \text{ ion at } m/z\ 262)\}}\\&\quad\times 100\% = 14\%\end{aligned}$$

[3′,5′,5′,7′,7′,7′-D$_6$, 4′-^{18}O]ABA methyl ester

[3′,5′,5′,7′,7′,7′-D$_6$]ABA methyl ester (2 mg) containing 97%, 93% and 96% deuterium at H-3′, H-5′ and H-7′, respectively, was dissolved in a mixture of 15 µl of acetic anhydride and 45 µl of H$_2$^{18}O, and the solution was left at room temperature for 16 h. Two milliliters of toluene was added, and the solution was concentrated to give [3′,5′,5′,7′,7′,7′-D$_6$,4′-^{18}O]ABA methyl ester (2 mg). ^{1}H NMR (CDCl$_3$) : δ 1.01 (3H, s, H-9′), 1.11 (3H, s, H-8′), 1.89 (4.5% of 3H, br.s), 2.01 (3H, d, $J = 1.1$ Hz, H-6), 2.29 (7% of 1H, br.s), 2.46 (7% of 1H, br.s), 3.71 (3H, s, 1-OCH$_3$), 5.76 (1H, s, H-2), 5.94 (3% of 1H, br.s), 6.15 (1H, d, $J = 16.2$ Hz, H-5), 7.88 (1H, d, $J = 16.2$ Hz, H-4). EI–MS (probe) 70 eV: m/z (rel. int.): 286 $[M+8]^+$ (4), 285 $[M+7]^+$ (1), 284 $[M+6]^+$ (2), 283 $[M+5]^+$ (0.5), 268 (3), 267 (2), 266 (3), 265 (2), 264 (0.5), 254 (4), 228 (6), 213 (3), 196 (100), 195 (20), 194 (40), 193 (7), 168 (21), 167 (12), 166 (13), 165 (6), 140 (10), 139 (8), 138 (13), 125 (34), 112 (8), 95 (6). The ^{18}O content was calculated from relative intensities of the molecular ions and found to be 69%, and the deuterium contents at H-3′, H-5′ and H-7′ were unchanged. The percentages of $[M+8-DH^{18}O]^+$ and $[M+8-DHO]^+$ ions in the dehydrated ions formed by loss of 4′-^{18}O and 1′-OH, respectively, were calculated using the following equations:

$$\begin{aligned}&\text{Percent } [M+8-DH^{18}O]^+ \text{ ion in the dehydrated}\\&\qquad\text{ion formed by loss of } 4'\text{-}^{18}O\\&= \frac{5\%\ (\text{percent } [M+8-DH^{18}O]^+ \text{ ion at } m/z\ 265)}{\{27\%\ (\text{percent } [M+8-H_2{}^{18}O]^+ \text{ ion at } m/z\ 266) + 5\%\ (\text{percent } [M+8-DH^{18}O]^+ \text{ ion at } m/z\ 265)\}}\\&\quad\times 100\% = 16\%\end{aligned}$$

$$\begin{aligned}&\text{Percent } [M+8-DHO]^+ \text{ ion in the dehydrated}\\&\qquad\text{ion formed by loss of } 1'\text{-OH}\\&= \frac{23\%\ (\text{percent } [M+8-DHO]^+ \text{ ion at } m/z\ 267)}{\{45\%\ (\text{percent } [M+8-H_2O]^+ \text{ ion at } m/z\ 268) + 23\%\ (\text{percent } [M+8-DHO]^+ \text{ ion at } m/z\ 267)\}}\\&\quad\times 100\% = 34\%\end{aligned}$$

RESULTS AND DISCUSSION

C-4′ of ABA methyl ester was labeled with ^{18}O from H$_2$^{18}O by hydration under the acidic condition. Introduction of ^{18}O into C-4′ was confirmed by the NMR analysis of [4′-^{17}O]ABA methyl ester which was prepared with H$_2$^{17}O by the same method as that used for the preparation of [4′-^{18}O]ABA methyl ester. Typical ^{17}O NMR chemical shift ranges are −50 to +140 ppm for alkoxy oxygen in ester group, −40 to +100 ppm for oxygen at hydroxyl group, +290 to +380 ppm for carbonyl oxygen in ester group, and +470 to +615 ppm for oxygen at ketone.[8] The ^{17}O NMR spectrum of the ^{17}O-labeled ABA methyl ester showed a broad singlet signal at 541.9 ppm assignable to the O-4′, showing that oxygen only at C-4′ was labeled under the acidic condition.

[4′-^{18}O]ABA methyl ester (Fig. 2) gave molecular ions corresponding to $[M+2]^+$ and $[M]^+$ at m/z 280 and 278, respectively, in a 92 : 8 ratio (Fig. 1b, Table 1), indicating that the ^{18}O content was 92%. Its dehydrated ions $[M+2-H_2O]^+$ and $[M+2-H_2{}^{18}O]^+/[M-H_2O]^+$ were observed at m/z 262 and 260, respectively, in a 62 : 38 ratio. This showed that the dehydrated ion of ABA methyl ester was formed not only by loss of the 1′-hydroxyl group but also by loss of the 4′-carbonyl oxygen. The percentages of dehydrated ions $[M+2-H_2O]^+$ and $[M+2-H_2{}^{18}O]^+$ were calculated to be 67% and 33%, respectively, using the following equations:

$$\begin{aligned}&\text{Percent } [M+2-H_2O]^+ \text{ ion}\\&= \frac{62\%\ (\text{percent dehydrated ion at } m/z\ 262)}{92\%\ (\text{percent molecular ion at } m/z\ 280)}\\&\quad\times 100\% = 67\%\end{aligned}$$

$$\begin{aligned}&\text{Percent } [M+2-H_2{}^{18}O]^+ \text{ ion}\\&= \frac{\{38\%\ (\text{percent dehydrated ion at } m/z\ 260) - 8\%\ (\text{percent } [M-H_2O]^+ \text{ ion at } m/z\ 260)\}}{92\%\ (\text{percent molecular ion at } m/z\ 280)}\\&\quad\times 100\% = 33\%\end{aligned}$$

These percentages in the mass spectra of the other scans were also calculated, and the mean of dehydrated ions $[M+2-H_2O]^+$ and $[M+2-H_2{}^{18}O]^+$ of three scans were 67 ± 3% and 33 ± 3%, respectively. This meant that 33% of

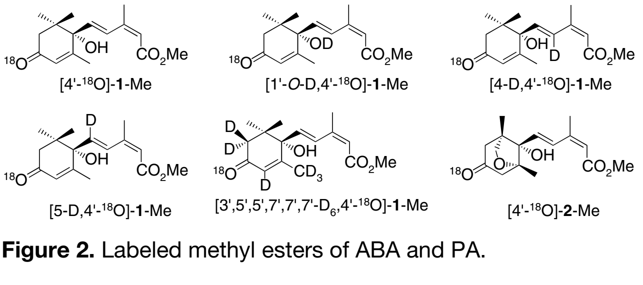
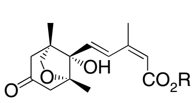


Figure 2. Labeled methyl esters of ABA and PA.

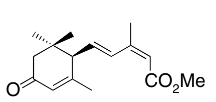
the dehydrated ion was derived from loss of the 4′-carbonyl oxygen and 67% from loss of the 1′-hydroxyl group. The difference between the relative intensities of the dehydrated ions at m/z 262 and 260 can be used to distinguish the ^{18}O-labeled position between C-1′ and C-4′ of ABA methyl ester.[5] If C-1′ is labeled with ^{18}O, the relative intensities of the dehydrated ions at m/z 262 and 260 should be 33% and 67%, respectively.

PA (**2**) is a natural metabolite of ABA, and formed by 8′-hydroxylation and isomerization of ABA in plants.[9] PA methyl ester gives an $[M]^+$ ion at m/z 294 and an $[M - H_2O]^+$ ion at m/z 276 (Fig. 1c, Table 1). Dehydration by loss of a carbonyl oxygen was observed in PA methyl ester also. [4′-^{18}O]PA methyl ester (Fig. 2) showing molecular ions $[M + 2]^+$ and $[M]^+$ at m/z 296 and 294, respectively, in a 94 : 6 ratio (Fig. 1d, Table 1), gave dehydrated ions $[M + 2 - H_2O]^+$ and $[M + 2 - H_2{}^{18}O]^+/[M - H_2O]^+$ at m/z 278 and 276, respectively, in a 20 : 80 ratio. This showed that PA methyl ester gave the dehydrated ion derived from loss of the 4′-carbonyl oxygen in addition to that derived from loss of the 1′-hydroxyl group. The percentages of dehydrated ions $[M + 2 - H_2O]^+$ and $[M + 2 - H_2{}^{18}O]^+$ derived from the molecular ion $[M + 2]^+$ were calculated in the same manner as that described for [4′-^{18}O]ABA methyl ester to be 21 ± 2% and 79 ± 2%, respectively. This result indicated that the dehydrated ion of PA methyl ester was mainly formed by loss of the 4′-carbonyl oxygen, and dehydration by loss of the 1′-hydroxyl group was minor.

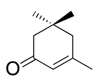
ABA and PA have a tertiary hydroxyl group at C-1′. In order to obtain clear evidence of the elimination of the 4′-carbonyl oxygen in the absence of the tertiary hydroxyl group, we examined EI mass spectra of 1′-deoxy-ABA methyl ester (**3**) and isophorone (**4**) which do not possess a tertiary hydroxyl group at the position corresponding to C-1′ of ABA and PA methyl esters. Unexpectedly, compounds **3** and **4** did not show dehydrated ions at m/z 244 and 120, respectively. This finding revealed that the 1′-hydroxyl group was necessary for dehydration by loss of the 4′-carbonyl oxygen of ABA and PA methyl esters. The 1′-hydroxyl hydrogen may be eliminated with the 4′-carbonyl oxygen as water. We examined this possibility with [1′-*O*-D,4′-^{18}O]ABA methyl ester (Fig. 2). [1′-*O*-D,4′-^{18}O]ABA methyl ester showed a dehydrated ion $[M + 3 - DH{}^{18}O]^+$ at 25% of all the dehydrated ions derived from the molecular ion $[M + 3]^+$ at m/z 281 (Table 2). The percentage of $[M + 3 - DH{}^{18}O]^+$ ion in the dehydrated ions derived from $[M + 3]^+$ ion by loss of 4′-^{18}O was 81% (for the calculation, see the experimental section). This finding showed that one hydrogen atom of water derived from the 4′-carbonyl oxygen was the 1′-hydroxyl hydrogen, confirming that the 1′-hydroxyl group was necessary for dehydration by loss of the 4′-carbonyl oxygen.



2: R = H
2-Me: R = Me



3



4

Another hydrogen atom involved in dehydration by loss of the 4′-carbonyl oxygen seemed to be derived from C-4 or -5 or -3′ or -5′ or -7′, since these hydrogen atoms were eliminated to give the dehydrated ion of ABA methyl ester.[4] [4-D,4′-^{18}O], [5-D,4′-^{18}O] and [3′,5′,5′,7′,7′,7′-D$_6$,4′-^{18}O]ABA methyl esters (Fig. 2) were prepared, and their EI–MS were analyzed. Relative intensities and compositions of molecular and dehydrated ions of these methyl esters are summarized in Table 2. [4-D,4′-^{18}O]ABA methyl ester gave the dehydrated ion $[M + 3 - DH{}^{18}O]^+$ at m/z 260 at 9% of all the dehydrated ions derived from the molecular ion $[M + 3]^+$

Table 1. Relative intensities[a] and compositions[b] of molecular and dehydrated ions of nonlabeled and ^{18}O-labeled ABA and PA methyl esters

Nonlabeled ABA methyl ester				[4′-^{18}O]ABA methyl ester			
m/z	Ion species	rel. int. (%)	Comp. (%)	m/z	Ion species	rel. int. (%)	Comp. (%)
278	$[M]^+$	3.4	100	280	$[M + 2]^+$	3.4	92
				278	$[M]^+$	0.3	8
260	$[M - H_2O]^+$	6.1	100	262	$[M + 2 - H_2O]^+$	5.6	62 (67)[c]
				260	$[M + 2 - H_2{}^{18}O]^+$	3.5	30 (33)[c]
					$[M - H_2O]^+$		8
Nonlabeled PA methyl ester				**[4′-^{18}O]PA methyl ester**			
m/z	Ion species	rel. int. (%)	Comp. (%)	m/z	Ion species	rel. int. (%)	Comp. (%)
294	$[M]^+$	24.1	100	296	$[M + 2]^+$	45.7	94
				294	$[M]^+$	3.1	6
276	$[M - H_2O]^+$	21.1	100	278	$[M + 2 - H_2O]^+$	6.4	20 (21)[c]
				276	$[M + 2 - H_2{}^{18}O]^+$	25.6	74 (79)[c]
					$[M - H_2O]^+$		6

[a] Relative intensities were corrected by natural ^{13}C-, ^{2}H- and ^{17}O-isotopic abundance.

[b] Compositions of relative intensities of each ion group.

[c] Compositions of dehydrated ions $[M + 2 - H_2O]^+$ and $[M + 2 - H_2{}^{18}O]^+$.

Table 2. Relative intensities[a] and compositions[b] of molecular and dehydrated ions of labeled ABA methyl esters

[1′-*O*-D,4′-^{18}O]ABA methyl ester				[4-D,4′-^{18}O]ABA methyl ester				[5-D,4′-^{18}O]ABA methyl ester				[3′,5′,5′,7′,7′,7′-D$_6$,4′-^{18}O]ABA methyl ester			
m/z	Ion species	rel. int. (%)	Comp. (%)	*m/z*	Ion species	rel. int. (%)	Comp. (%)	*m/z*	Ion species	rel. int. (%)	Comp. (%)	*m/z*	Ion species	rel. int. (%)	Comp. (%)
281	$[M+3]^+$	2.8	67	282	$[M+4]^+$	0.2	4	281	$[M+3]^+$	2.6	76	286	$[M+8]^+$	3.4	56
280	$[M+2]^+$	0.9	21	281	$[M+3]^+$	3.9	75	280	$[M+2]^+$	0.2	6	285	$[M+7]^+$	0.8	13
279	$[M+1]^+$	0.5	12	280	$[M+2]^{+}$[d]	0.5	9	279	$[M+1]^+$	0.6	18	284	$[M+6]^+$	1.4	23
					$[M+2]^{+}$[e]		1					283	$[M+5]^+$	0.5	8
				279	$[M+1]^+$	0.6	12								
263	$[M+3-H_2O]^+$	2.7	21 (31)[c]	264	$[M+4-H_2O]^+$	0.3	3	263	$[M+3-H_2O]^+$	3.1	42 (55)[c]	268	$[M+8-H_2O]^+$	2.3	25 (45)[f]
262	$[M+3-DHO]^+$	3.9	25 (37)[c]	263	$[M+3-H_2O]^+$	1.7	18 (24)[c]	262	$[M+3-DHO]^+$	0.8	7 (9)[c]	267	$[M+8-DHO]^+$	1.9	13 (23)[f]
	$[M+2-H_2O]^+$		14	262	$[M+4-H_2{}^{18}O]^+$	4.0	1		$[M+2-H_2O]^+$		4		$[M+7-H_2O]^+$		8
261	$[M+3-H_2{}^{18}O]^+$	1.4	4 (6)[c]		$[M+3-DHO]^+$		34 (45)[c]	261	$[M+3-H_2{}^{18}O]^+$	2.8	23 (30)[c]	266	$[M+8-H_2{}^{18}O]^+$	3.0	15 (27)[f]
	$[M+1-H_2O]^+$		5		$[M+2-H_2O]^{+}$[d]		6		$[M+1-H_2O]^+$		15		$[M+7-DHO]^+$		1
260	$[M+3-DH^{18}O]^+$	2.9	17 (25)[c]		$[M+2-H_2O]^{+}$[e]		1	260	$[M+3-DH^{18}O]^+$	0.7	4 (5)[c]		$[M+6-H_2O]^+$		17
	$[M+2-H_2{}^{18}O]^+$		7	261	$[M+3-H_2{}^{18}O]^+$	2.0	16 (21)[c]		$[M+2-H_2{}^{18}O]^+$		2	265	$[M+8-DH^{18}O]^+$	1.5	3 (5)[f]
	$[M+1-DHO]^+$		7		$[M+1-H_2O]^+$		5		$[M+1-DHO]^+$		3		$[M+7-H_2{}^{18}O]^+$		1
				260	$[M+3-DH^{18}O]^+$	1.6	7 (9)[c]						$[M+6-DHO]^+$		6
					$[M+2-H_2{}^{18}O]^{+}$[d]		3						$[M+5-H_2O]^+$		6
					$[M+1-DHO]^+$		7					264	$[M+7-DH^{18}O]^+$	0.5	3
													$[M+5-DHO]^+$		2

[a] Relative intensities were corrected by natural ^{13}C-, ^{2}H- and ^{17}O-isotopic abundance.

[b] Compositions of relative intensities of each ion group.

[c] Compositions of dehydrated ions $[M+3-H_2O]^+$, $[M+3-DHO]^+$, $[M+3-H_2{}^{18}O]^+$ and $[M+3-DH^{18}O]^+$.

[d] Molecular ion labeled with ^{18}O or dehydrated ions derived from this molecular ion.

[e] These ions were labeled with two deuterium atoms, since [4-D,4′-^{18}O]ABA methyl ester was slightly labeled at C-2 with deuterium in addition to C-4.

[f] Compositions of dehydrated ions $[M+8-H_2O]^+$, $[M+8-DHO]^+$, $[M+8-H_2{}^{18}O]^+$ and $[M+8-DH^{18}O]^+$.

Table 3. Percentages of hydrogen atoms involved in dehydration by loss of the 4′-carbonyl oxygen and 1′-hydroxyl group in EI–MS of ABA methyl ester

			Percentage of hydrogen atoms involved in each dehydrated ion[b]					
Eliminating groups involved in dehydration	Percent[a,b]	Order of hydrogen	1′-OH	4-H	5-H	3′,5′,7′-H_6	Others (%)	Total (%)
4′-Carbonyl oxygen	33 ± 3%	First	81 ± 0%	–	–	–	19%	100%
		Second	–	30 ± 1%	14 ± 1%	15 ± 1%	41%	100%
1′-Hydroxyl group	67 ± 3%	–	–	68 ± 3%	14 ± 2%	35 ± 1%	–	117%
Total	100%	–	–	–	–	–	–	–

[a] Percentage of each dehydrated ion in the total dehydrated ions.

[b] Percentages shown are the mean value ± SD of three scans.

at m/z 281, and this ion consisted of 30% of the dehydrated ions derived from loss of 4′-^{18}O (for the calculation, see the experimental section). This result showed involvement of 4-hydrogen in dehydration by loss of the 4′-carbonyl oxygen. The dehydrated ion $[\mathrm{M}+3-\mathrm{DHO}]^+$ at m/z 262 corresponded to the dehydrated ion formed by loss of the 1′-hydroxyl group and deuterium atom at C-4. This ion accounted for 65% of the dehydrated ions derived from the loss of 1′-OH, confirming the involvement of 4-hydrogen in the dehydration by loss of the 1′-hydroxyl group.[4] [5-D,4′-^{18}O]ABA methyl ester showed the dehydrated ion $[\mathrm{M}+3-\mathrm{DH}^{18}\mathrm{O}]^+$ at m/z 260 in 5% of all the dehydrated ions derived from the molecular ion $[\mathrm{M}+3]^+$ at m/z 281, and the percentage of $[\mathrm{M}+3-\mathrm{DH}^{18}\mathrm{O}]^+$ ion in the dehydrated ions derived from loss of 4′-^{18}O was 14% (for the calculation, see the experimental section). This result showed that the involvement of 5-hydrogen in the dehydration by loss of the 4′-carbonyl oxygen was small. Nine percent of the dehydrated ion $[\mathrm{M}+3-\mathrm{DHO}]^+$ in all the dehydrated ions derived from the molecular ion $[\mathrm{M}+3]^+$ was detected at m/z 262. The $[\mathrm{M}+3-\mathrm{DHO}]^+$ ion was produced at 14% in the dehydrated ions derived from loss of 1′-OH. [3′,5′,5′,7′,7′,7′-D_6,4′-^{18}O]ABA methyl ester gave the dehydrated ion $[\mathrm{M}+8-\mathrm{DH}^{18}\mathrm{O}]^+$ at m/z 265 in 5% of all the dehydrated ions derived from the molecular ion $[\mathrm{M}+8]^+$ at m/z 286, and the percentage of $[\mathrm{M}+8-\mathrm{DH}^{18}\mathrm{O}]^+$ ion in the dehydrated ions formed by loss of 4′-^{18}O was 16% (for the calculation, see the experimental section). This result showed that 3′,5′,7′-hydrogen atoms were partially involved in dehydration by loss of the 4′-carbonyl oxygen. [3′,5′,5′,7′,7′,7′-D_6,4′-^{18}O]ABA methyl ester showed 23% of $[\mathrm{M}+8-\mathrm{DHO}]^+$ ion at m/z 267 of all the dehydrated ions derived from the molecular ion $[\mathrm{M}+8]^+$, revealing that 3′,5′,7′-hydrogen atoms were eliminated with the 1′-hydroxyl group also. $[\mathrm{M}+8-\mathrm{DHO}]^+$ ion accounted for 34% of the dehydrated ions derived from loss of 1′-OH. We could not examine the contribution of each hydrogen atom at C-3′, -5′ or -7′ to the dehydration because of the difficulty with selective deuteration at these carbons.

Percentage of hydrogen atoms involved in dehydration by loss of the 4′-carbonyl oxygen and 1′-hydroxyl group are summarized in Table 3. The first hydrogen atom eliminated with the 4′-carbonyl oxygen as water was 1′-hydroxyl hydrogen, and the percentage was 81%. The second hydrogen atom eliminated with the 4′-carbonyl oxygen as water was derived from the hydrogen atom at C-4 or -5 or -3′ or 5′ or 7′, and the sum of the percentages of the second hydrogen atoms was 59%. The origin of the residual 19% of the first hydrogen and 41% of the second hydrogen atoms could not be identified in this experiment. The hydrogen atom eliminated with the 1′-hydroxyl group as water was derived from C-4 or -5 or -3′ or 5′ or 7′ as well as the second hydrogen eliminated with the 4′-carbonyl oxygen. The sum of percentages of hydrogen atoms eliminated with the 1′-hydroxyl group was 117%. This would be due to analytical errors, and the sum should be close to 100%, suggesting that other hydrogen atoms were not involved in dehydration by loss of the 1′-hydroxyl group.

We propose the formation mechanism of the dehydrated ion derived from loss of the 4′-carbonyl oxygen, and 1′-hydroxyl hydrogen and 4-hydrogen on the basis of the above results (Fig. 3). The first step of the fragmentation is ionization at the 1′-hydroxyl group to give the ion radical **5**. The proton at 1′-oxygen of **5** should migrate to 4′-carbonyl oxygen, so that it may be lost with the 4′-carbonyl oxygen. This migration needs the proximity of these two groups, and seems to proceed by a similar mechanism to that in dehydration by loss of the 4′-hydroxyl groups in EI–MS of 1′,4′-*trans*- and 1′,4′-*cis*-diol-ABA methyl esters.[4] The bond fission between C-1′-C-6′ of the ion radical **5** gives **6**, and bond rotation at C-3′-C-4′ of the ion radical **6** forms **7**. In the ion radical **7**, 1′-hydroxyl hydrogen is close to 4′-oxygen, and the proton migrates from the 1′-hydroxyl group to 4′-oxygen to give the ion radical **8**. Successively, migration of double bonds in **8** forms the ion radical **9**. Migration of protons from C-5 to C-4 and from C-7′ to C-5 following proton migration from C-4 to 1′-oxygen gives the ion radical **10**. A proton at 1′-oxygen of the ion radical **10**, which is derived from C-4, migrates to 4′-oxygen to give the ion radical **11**, and then H_2O at C-4′ of **11** is eliminated to give the dehydrated ion **12**. Cyclization of the ion radical **12** by attack of an unpaired ion at C-6′ to C-1′ gives the dehydrated ion **13** with an ABA-like skeleton. The dehydrated ion **13** may be converted to other resonance forms. Dehydration by loss of the 4′-carbonyl oxygen with 1′-hydroxyl hydrogen and 5-hydrogen seems to occur by a similar mechanism to that with 1′-hydroxyl hydrogen and 4-hydrogen. If the bond at C-5-C-1′ of the ion radical **9** is rotated, the distance between 5-hydrogen and 1′-oxygen becomes closer. Migration of a proton at C-5 to 1′-oxygen and of a proton at C-7′ to C-5

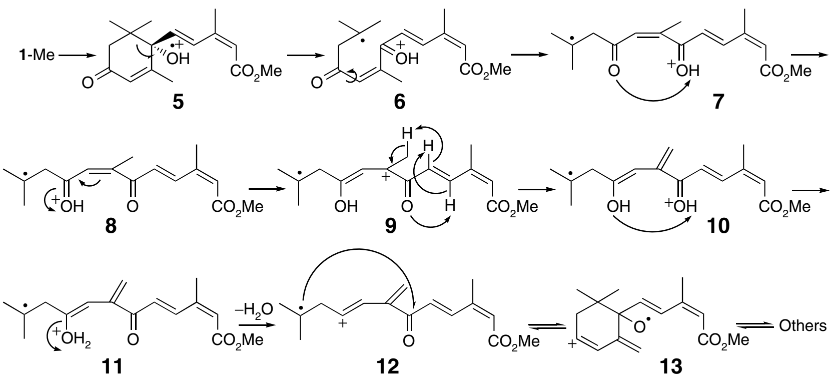


Figure 3. Dehydration by loss of the 4′-carbonyl oxygen with 1′-hydroxyl hydrogen and 4-hydrogen.

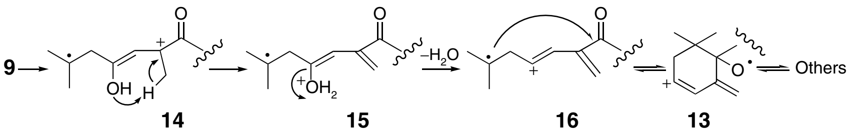


Figure 4. Dehydration by loss of the 4′-carbonyl oxygen with 1′-hydroxyl hydrogen and 7′-hydrogen.

forms the ion radical **10**, and the subsequent pathway is the same as that shown in Fig. 3. The contribution of each hydrogen atom at C-3′, -5′ or -7′ to dehydration by loss of the 4′-carbonyl oxygen is unknown, as described above. However, 7′-hydrogen is likely to be eliminated with the 4′-carbonyl oxygen, since the distance between 4′-oxygen and 7′-hydrogen decreases if a 180° rotation occurs around the C-2′-C-3′ bond of **9**. The structure of the ion radical **14** formed by this bond rotation is shown in Fig. 4. After migration of a proton at C-7′ to 4′-oxygen in **14** to form the ion radical **15**, elimination of the H_2O molecule from **15** gives the ion radical **16**. The ion radical **16** is cyclized by the attack of an unpaired ion at C-6′ to C-1′ to give the ion radical **13**, and then the ion may be converted to other resonance forms. The possibilities of migration of protons in the ion radicals **9** and **14** and of existence of positive electric charge on the double bonds in the ion radicals **12, 13**, and **16** appear to be small. However, these structures and structural exchanges could not be avoided to explain our experimental results. In these pathways of dehydration, the 1′-hydroxyl group is not only a source of a proton for H_2O but also a mediator for the proton migration from C-4 or -5 to 4′-carbonyl oxygen. This dehydration mechanism may apply to dehydration by loss of the 4′-carbonyl oxygen of PA methyl ester.

Dehydration in EI–MS usually occurs with elimination of a hydroxyl group,[10] so dehydration by loss of the carbonyl oxygen is an interesting fragmentation. Dehydration by loss of the carbonyl oxygen is known to occur in some aliphatic ketones and acyclic aldehyde, and in *o*-methoxybenzaldehyde, *o*-methoxyacetophenone, *o*-methoxybenzophenon, and *peri*-methoxyquinone.[11,12] As the mechanism for dehydration of these aromatic compounds, migration of a proton at a methoxy group to the carbonyl oxygen by a proximity effect is also suggested.[13] In these compounds, the hydrogen atom of the methoxy group and oxygen atom of the carbonyl group are close enough to cause a proximity effect. For ABA methyl ester, the proximity effect for loss of the carbonyl oxygen as water may be achieved by changes of the skeleton, including bond fission and rotation. This would be the first case for dehydration by loss of the carbonyl oxygen in nonaromatic and non-quinone-type compounds. We should remind ourselves that the presence of a dehydrated ion does not always mean the presence of a hydroxyl group and sometimes means the presence of a carbonyl group. The positive CI mass spectrum of ABA methyl ester gives a dehydrated ion at m/z 261 $[MH - H_2O]^+$ as the base peak in addition to a molecular ion $[MH]^+$ at m/z 279.[14] The oxygen involved in the dehydration of ABA methyl ester has been shown to be derived from the 1′-hydroxyl group, not from the 4′-carbonyl oxygen. The 1′-hydroxyl group would be lost with a proton derived from a reagent gas to give the dehydrated ion in positive CI-MS. Dehydration by loss of the 4′-carbonyl oxygen of ABA methyl ester would be a fragmentation characteristic of EI–MS.

CONCLUSIONS

Detection of a dehydrated ion in EI–MS is often used to prove the occurrence of a hydroxyl group in organic compounds, and a dehydration mechanism derived from the elimination of a hydroxyl group is usually proposed to explain the formation of the dehydrated ion. However, the dehydrated ion is not always derived from the elimination of a hydroxyl group, and involvement of carbonyl oxygen in dehydration is not restricted to aromatic and quinone-type compounds. We have shown here that 34% of the dehydrated ion of ABA methyl ester is derived from the elimination of the 4′-carbonyl oxygen and hydrogen atoms at the 1′-hydroxyl group, and mainly at C-4. For PA methyl ester, the percentage of the dehydrated ion derived from elimination of the 4′-carbonyl oxygen is up to 79%. There are many natural compounds having carbonyl and hydroxyl groups. We should consider the involvement of a carbonyl group in the formation of dehydrated ions, and analyze any compound labeled with ^{18}O at carbonyl oxygen when involvement of carbonyl oxygen in dehydration is plausible. We have proposed a mechanism for dehydration derived from the elimination of the 4′-carbonyl oxygen in ABA methyl ester, but more examples may be necessary to find a rule for dehydration derived from the elimination of carbonyl oxygen.

REFERENCES

1. Leung J, Giraudat J. Abscisic acid signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1998; **49**: 199.
2. Hartung W, Gimmler H. A stress physiological role for abscisic acid (ABA) in lower plants. *Prog. Bot.* 1994; **55**: 157.
3. Gray RT, Mallaby R, Ryback G, Williams VP. Mass spectra of methyl abscisate and isotopically labeled analogues. *J. Chem. Soc. Perkin Trans. II* 1974; 919.
4. Hirai N, Okamoto M, Fujimura M, Ichiyama T, Todoroki Y, Ohigashi H, Takeda N, Yoshizumi H, Tatematsu A. Mechanism of formation of dehydrated ions from abscisic acid and its 1′,4′-diol methyl esters in electron ionization mass spectrometry. *J. Mass Spectrom. Soc. Jpn.* 2000; **48**: 8.
5. Inomata M, Hirai N, Yoshida R, Ohigashi H. The biosynthetic pathway to abscisic acid *via* ionylideneethane in the fungus *Botrytis cinerea*. *Phytochemistry* 2004; **65**: 2667.
6. Hirai N, Kondo S, Ohigashi H. Deuterium-labeled phaseic acid and dihydrophaseic acids for internal standards. *Biosci. Biotechnol. Biochem.* 2003; **67**: 2408.
7. Todoroki Y, Hirai N, Koshimizu K. Synthesis and biological activity of 1′-deoxy-1′-fluoro- and 8′-fluoroabscisic acids. *Phytochemistry* 1995; **40**: 633.
8. Baumstark AL, Boykin DW. ^{17}O NMR spectroscopy: applications to structural problems in organic chemistry. *Adv. Oxygenated Process* 1991; **3**: 141.
9. Zeevaart JAD. Abscisic acid metabolism and its regulation. In *Biochemistry and Molecular Biology of Plant Hormones*, Hooykaas PJJ, Hall MA, Libbenga KR (eds). Elsevier Science: Amsterdam, 1999; 189.
10. Budzikiewicz H, Djerassi C, Williams DH. *Mass Spectrometry of Organic Compounds*, Holden-Day: San Francisco, 1967; 690.
11. Yeo ANH, Williams DH. The loss of water from molecular ions of aliphatic ketones in electron-impact mass spectra. *Org. Mass Spectrom.* 1969; **2**: 331.
12. Bowie JH. Reactions of specific functional groups. In *Mass Spectrometry-Volume 1*, Williams DH (ed). The Chemical Society Burlington House: London, 1971; 91.
13. Bowie JH, White PY. Electron impact studies. Part XXXIX. Proximity effect in the mass spectra of aromatic carbonyl compounds containing adjacent methoxy-substituents. *J. Chem. Soc. B* 1969; 89.
14. Netting AG, Milborrow BV, Vaughan GT. The fragmentation of methyl abscisate and its 2*E* isomer in methane positive and negative chemical ionization mass spectrometry. *Biomed. Environ. Mass Spectrom.* 1988; **15**: 375.