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CHIRAL SYNTHESIS AND STOMATAL CLOSURE ACTIVITIES OF γ-*PSEUDO*- AND DIHYDRO-ABSCISIC ACIDS*

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Key Word Index—*Cercospora cruenta*; Hyphomycetes; *Commelina communis*; Commelinaceae; abscisic acid; positional isomer; chiral synthesis; stomatal closure.

Abstract—Analogues of abscisic acid (ABA) were prepared from chiral cyclohexanone building blocks. Synthetic (1′*R*, 3′*S*)-1′, 3′-γ-dihydroxy-γ-ionylideneacetic acid and (*R*)-1′-hydroxy-3′-oxo-γ-ionylideneacetic acid (tentatively named γ-*pseudo*-ABA) provided authentic data for new metabolities isolated from *Cercospora cruenta*. Both enantiomers of 2′, 3′-dihydroABA were also prepared. Only the (1′*S*, 2′*S*)-isomer of 2′, 3′-dihydroABA induced substantial closure of stomata on the epidermal strips of *Commelina communis*.

INTRODUCTION

Several analogues of abscisic acid (ABA) are known as natural products and have been reported as being biologically active. For example, xanthoxin [1], abscisic aldehyde [2] and phaseic acid [3] have been found in higher plants, while 1′-deoxy-ABA [4, 5], 1′,4′-dihydroxy-α-ionylideneacetic acid (1′, 4′-dihydroxy-α-acid) [6] and 1′, 4′-dihydroxy-γ-acid [7] occur as fungal metabolites. Although nearly 90 gibberellins have been reported, including positional isomers [8, 9], ABA analogues should not be regarded as positional isomers, but instead as intermediates or catabolites on the ABA biosynthetic pathway. Here, (*R*)-5-(1′-hydroxy-2′, 2′, 6′-trimethyl-3′-oxo-5′-cyclohexen-1′-yl)-3-methyl-2,4-pentadienoic acid (1′-hydroxy-3′-oxo-α-acid, **1**), corresponding to the 5′-oxo-isomer of ABA, and (*R*)-5-(1′-hydroxy-2′, 2′-dimethyl-3′-oxo-6′-methylenecyclohexyl)-3-methyl-2,4-pentadienoic acid (1′-hydroxy-3′-oxo-γ-acid, **2**) are tentatively designated as α-*pseudo*-ABA and γ-*pseudo*-ABA, respectively (Fig. 1). No such positional isomers of ABA have been reported thus far, although their natural occurrence seems very likely. The isolation of plutidione (**3**) from a common Pakistani weed, *Pluchea arguta* [10], is reminiscent of the formation and oxidative degradation of **1** in this plant. On the other hand, 3′-hydroxy-γ-acid (**4a**) and 1′, 3′-dihydroxy-γ-acid (**4b**) were isolated from an ABA producing fungus, *Cercospora cruenta*. Moreover, administration of ^{14}C-labelled **4a** suggested the formation of **2** via **4b** in this fungus [11]. The main objective in this paper is to provide chiral **2** and **4b** (= **11**) as authentic specimens for the previous isolation work [11].

The chiral synthesis of 2′, 3′-dihydroABA (**5**) and its epimer was first reported by Lamb and Abrams [12]. High affinity of (1′*S*, 2′*S*)-**5** to ABA monoclonal antibody [13], germination inhibitory activity of (1′*S*, 2′*S*)-**5** [14] and biological transformation of ABA to (1′*S*, 2′*R*)-2′, 3′-dihydroABA (**6**) [15] were also reported. The stomatal closure activity of (±)-**5** was previously measured [16], but the significance of its stereochemistry was not established. A second aim of this paper is to examine the importance of the conjugated double bond at the 2′-position of ABA for its biological activity. Thus, the stomatal closure activity of (*S*)-ABA was compared with that of (*R*)-**2**, (1′*S*, 2′*S*) and (1′*R*, 2′*R*)-**5**.

RESULTS AND DISCUSSION

Synthesis of γ-pseudo*ABA* (**2**)

The α-methylene ketone **7a** was reacted with Grignard reagent prepared from (2*Z*)-3-methyl-2-penten-4-yn-1-yl tetrahydropyranyl (THP) ether to give a diastereomeric mixture of acetylenic alcohols, the *cis*-isomer **8a** and *trans*-isomer **9a**, in a ratio of 31 : 19, respectively (Fig. 2). The Grignard reagent prefers axial attack on the carbonyl group of **7a**. The THP ether of **8a** was next deprotected by acetic acid to give the *cis*-triol 8, and, in a similar manner, the *trans*-isomer **9a** was deprotected to **9**, which was subsequently shown to be a mixture of α- and γ-triols (41 : 59) by inspection of its ^{1}H NMR spectrum. The partial migration of the terminal double bond of **9** could be caused by severe steric hindrance from the 3′-THPoxy

*The carbon numbering of dihydroABA is based on original ABA nomenclature, whereas compounds related to γ-*pseudo*-ABA follow IUPAC nomenclature.

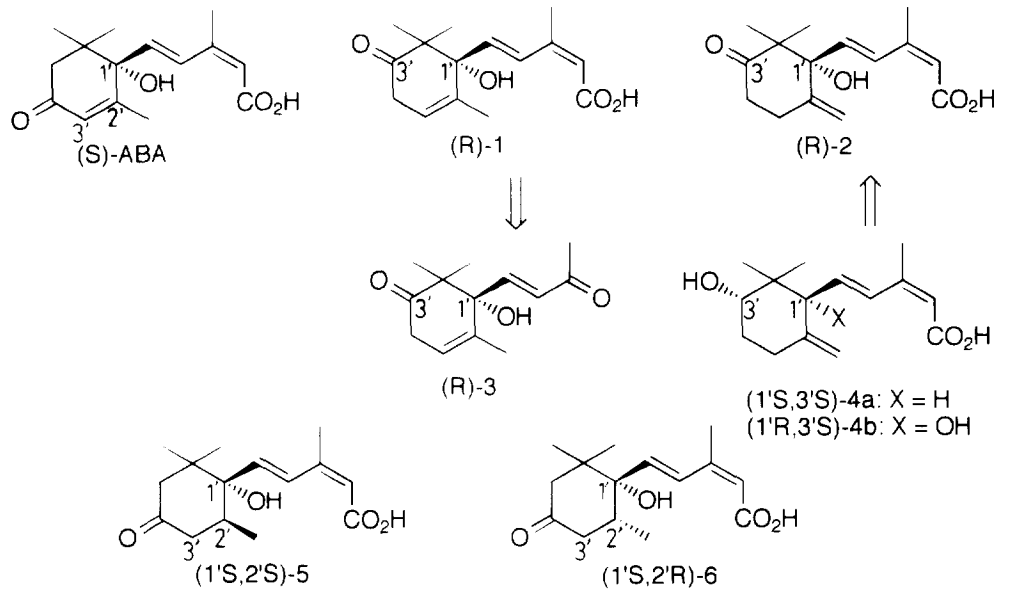

Fig. 1. (*S*)-ABA and its related compounds.

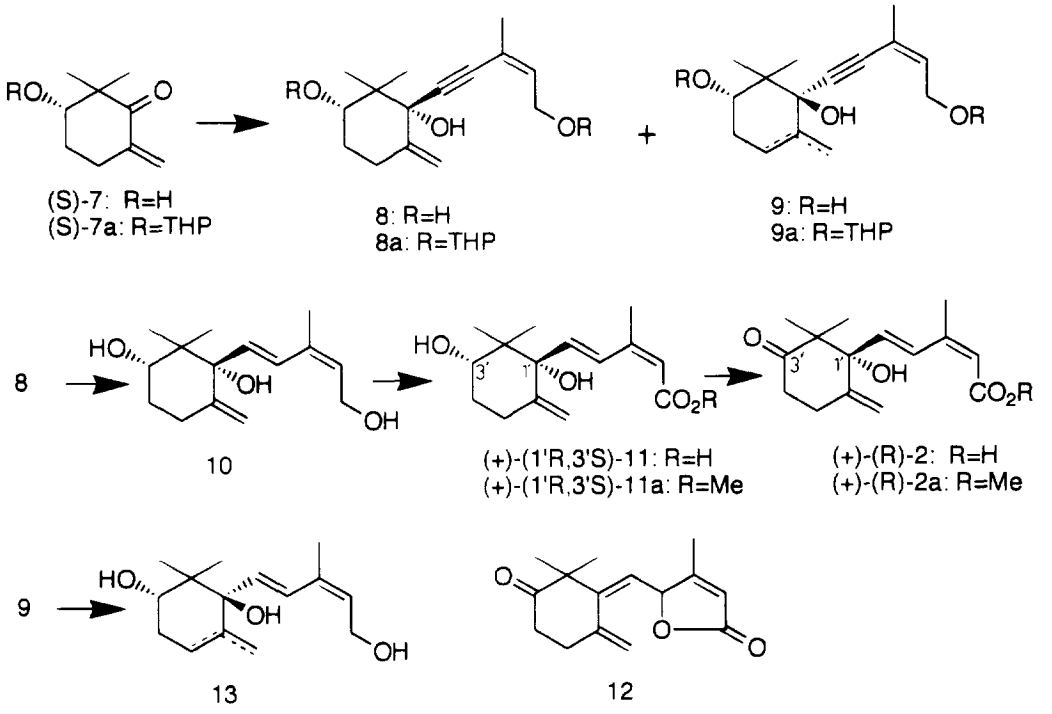

Fig. 2. Synthesis of (*R*)-γ-*pseudo*-ABA.

group on to the axial acetylenic side chain. Thus, the double bond isomerization would remove the A-strain effect [17] and permit an equatorial arrangement of the side chain. The acetylenic bond of **8** was reduced by sodium bis-(2-methoxyethoxy)aluminium hydride (Redal) to give the allylic alcohol **10**. Oxidation of 10 by MnO_2 gave aldehyde **10**, which was further reacted with NaCN in the presence of MnO_2 and methanol to afford the methyl ester **11a**. The positive Cotton effect (265 nm) of **11a** on the circular dichroic spectrum (265 nm) was indicative of a (1′*R*)-configuration, the same as naturally occurring (*S*)-ABA. Alkaline hydrolysis of **11a** gave free acid **11**. NOE difference spectra of **11** reconfirmed the (1′*R*, 3′*S*)-configuration (Fig. 3), which was identical to natural **4b** isolated from the culture broth of *C. cruenta* [11]. Irradiation of Hi (δ: 5.73) caused clear difference

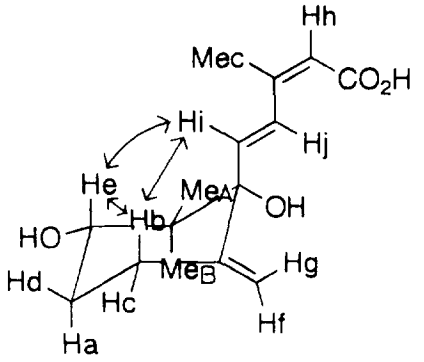

Fig. 3. ^{1}H NMR NOE of **11**.

signals on Hb (*ca* 5–8% enhancement, δ:1.80), He (7–10%, δ: 3.58), Me_A (6–8%, δ:0.92) and Me_C (7–9%, δ:2.07). Whereas irradiation of He affected Hb (9–13%), Hd (5–9%, δ:2.91) and Hi (5–8%). In a similar manner, irradiation of Ha affected Hc (5–9%, δ:2.70), Hd (11–13%) and Me_B (7–10%). These data indicated that **11** has a cyclohexane ring in chair conformation with an axial pentadienoic acid at the 1′-position and equatorial hydroxyl groups at 1′- and 3′-positions.

Treatment of **11a** with pyridinium chlorochromate (PCC) gave methyl γ-*pseudo*-ABA (**2a**), but subsequent alkaline hydrolysis caused the complete destruction of **2a**. The free acid **2** was finally obtained in a high yield by Dess–Martin oxidation [18] of **11**. When **2** was treated by PCC, a rearrangement of its *tert*-hydroxyl group first occurred, followed by auto-lactonization to give optically inactive γ-valerolactone (**12**) in low yield. The synthesis of antipodal **2** was also intended from **9a**, which was converted into **13** as a mixture of α- and γ-triols. However, the separation of each isomer was unsuccessful.

As reported previously [11], the natural occurrence of (*R*)-**2** was suggested from the fact that a substantial amount of (1′*S*, 3′*S*)-**4a** was isolated and converted into (1′*R*, 3′*S*)-**11**, but the isolation of **2** has not been successful, probably due to its small quantity and instability. The formation of **2** was merely suggested by TLC in the administration of ^{14}C-labelled **4a** [11]. The mobility of **2** on TLC or HPLC was close to that of ABA, but distinctly separate. In summary, new metabolites, (*R*)-**2**, (1′*R*, 3′*S*)-**11** and their methyl esters, were prepared stereoselectively.

Stability of pseudo-*ABA*

To prepare **1**, acid- or base-catalysed isomerization of **2** was examined. Surprisingly, rapid decomposition of **2** was always observed within a few hours, though a variety of catalysts were examined under various conditions. This fact indicates the extraordinary vulnerability of *pseudo*-ABA. It is deduced that the retro-Aldol reaction of the β-hydroxyketone moiety could cause ring opening and this would lead to the complete degradation of the original molecule. The identification of *pseudo*-ABA from a natural source has not been reported yet. Breakdown of *pseudo*-ABA during the isolation procedure seems plausible even under mild basic or acidic conditions.

Preparation of both epimers of dihydroABA (**5**)

The present synthetic routes to chiral **5** are fundamentally the same as those reported by Lamb and Abrams [12]. The reaction of chiral ketone **14a** [19] with lithium acetylide prepared from (2*Z*)-3-methyl-2-penten-4-yn-1-yl THP ether gave **15a** almost exclusively (Fig. 4). The preference for axial addition of acetylide to a carbonyl group has already been predicted [12], and the equatorial adduct has not been isolated. The following transformation of **15a** via **16** gave the synthetic enantiomer (1′*R*, 2′*R*)-**5**. The natural enantiomer (1′*S*, 2′*S*)-**5** was prepared from another chiral ketone **17** [19]. The exclusive axial addition of acetylide to a *tert*-butyldimethylsilyl derivative of **17** has been reported [12], and the same result was obtained for the THPoxy derivative **17a**.

The mobility of **5** was very close to that of ABA on TLC and HPLC, while the epimer **6** has an equatorial side chain and can be separated from ABA by TLC [15].

Stomatal closure activities

The biological activities of (*R*)-**2**, (1′*S*, 2′*S*)- and (1′*R*, 2′*R*)-**5** were tested on stomata of *Commelina communis* (Fig. 5). Although (*R*)-**2** was reported to be a strong inhibitor of radish germination [11], the effect on stomatal closure was very minor. This result suggests that the receptors for germination inhibition and stomatal closure have different requirements. Like a previous stomatal closure assay using (±)-**5** compared with (±)-ABA, the natural enantiomer (1′*S*, 2′*S*)-**5** showed about one-tenth the activity of (*S*)-ABA, while antipodal (1′*R*, 2′*R*)-**5** was almost inactive.

It is believed that ABA is rapidly catabolized to inactive phaseic acid in watered water-stressed plants, and that this conversion is triggered by a stereoselective enzymic hydroxylation [17]. However, the following cyclization to phaseic acid seems rather spontaneous and proceeds even by a simple chemical treatment [21]. Although it was expected that compounds such as **2** and **5** might be immune to such a catabolic process without the conjugated double bond at the 2′-position of ABA [22, 33], the stomatal closure activity of the tested compounds was inferior to that of (*S*)-ABA. In summary, it is suggested that the 2′-double bond of ABA is not only required for the catabolic mechanism, but is also as an important site for stomatal closure activity. It is also recognized that the correct stereochemistry at the 1′-position is a more important factor than the effect of the double bond. Preparation and biological tests of (*R*)-**1** are now in progress.

EXPERIMENTAL

Mps: uncorr. Optical rotations were measured with a JASCO DIP-4 spectrometer. CD and ORD spectra

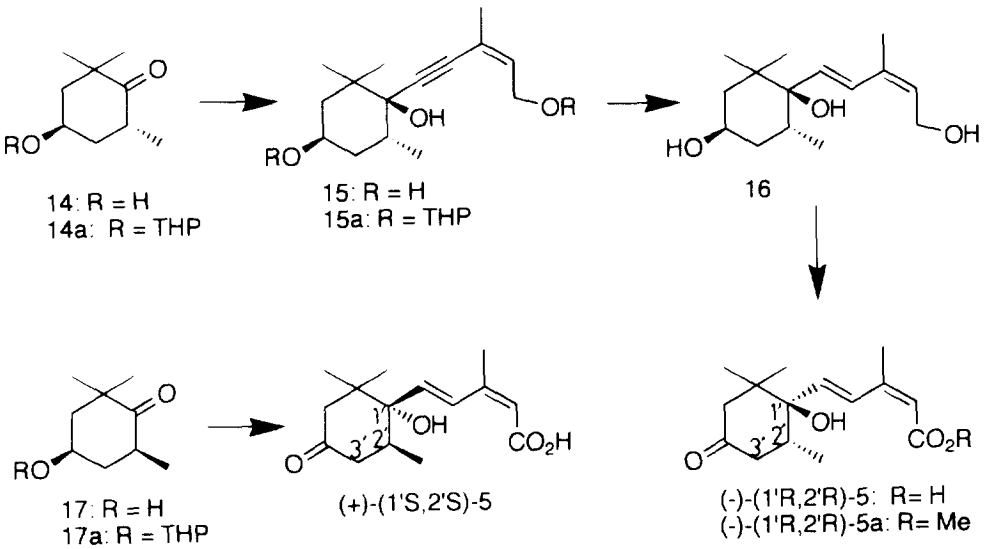

Fig. 4. Synthesis of both enantiomers of dihydroABA.

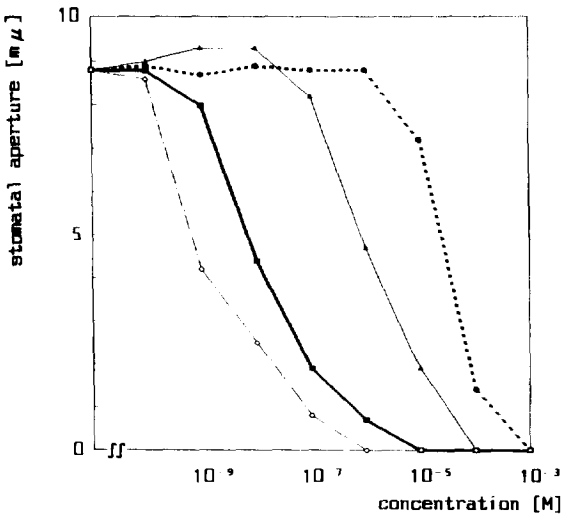

Fig. 5. Stomatal closure activities on isolated epidermis of *Commelina communis*. (*S*)-ABA (---◇---). (*R*)-γ-*pseudo*-ABA. **2** (—▲—); (1'*S*, 2'*S*) -2', 3'-dihydroABA. **5** (—■—); (1'*R*, 2'*R*) -**5** (---●---).

were recorded by a Diochrograph Mark III-J instrument and a Model ORD JASCO J-A 20, respectively. IR spectra were recorded on a JASCO IR-810 spectrometer. ^{1}H NMR was measured on a JEOL JNM FX-100 (100 MHz) or JEOL GSX-270 (270 MHz) spectrometer with TMS as int. standard. Mass spectra were recorded with a JEOL JMX HX-105 instrument (70 eV, 120–180°) or Carlo-Erba Irba instrument (70 eV, 180°). UV spectra were recorded on a Hitachi Model 124 spectrometer. HPLC analyses were attained using Partisil (250 × 4.5 mm) eluted with $CHCl_3$–MeCN–HOAc (45:5:1). CC and flash chromatography were performed by using silica gel (100–200 and 60 mesh, respectively). Prep. TLC was carried out on Merck 60 PF_{254} (0.7 mm thickness, 20 × 20 cm).

(−)-(*S*)-2,2-*Dimethyl*-6-*methylene*-3-*tetrahydropyranoxy*-1-*cyclohexanone* (**7a**). In the same manner as reported previously [17], (*S*)-2,2-Dimethyl-3-tetrahydropyranyloxy-1-cyclohexanone (7.30 g, 99%e.e) [24] was converted into the ketone **7a** (8.81 g, 72% yield); oil, $[\alpha]_D^{20}$ − 3.1° ($CHCl_3$, *c* 1.06). IR ν_{max}^{film} cm^{-1}: 3080, 1700, 1620, 1135, 1120, 1075, 1035. EIMS (GC) 70eV, *m/z* (rel. int.): 477 (2.7), 476 $[2M]^+$ (31), 393 (8), 392 $[2M - THP]^+$ (100), 308 (27), 290 (4). ^{1}H NMR (100 MHz, $CDCl_3$): δ1.13–1.24 (6H, signals at 1.13, 1.16, 1.24), 1.32–3.02 (12H, *m*), 3.48–3.72 (1H, *m*), 3.78–4.08 (1H, signals at 3.64, 3.79), 5.15 (1H, *br. s*), 5.73 (1H, *br. s*). Found: C, 70.51; H, 9.36. $C_{14}H_{22}O_3$ requires: C, 70.55; H, 9.31%.

(1'S, 3'S)- *and* (1'R, 3'S)-(Z)-5-(1'-*Hydroxy*-2', 2'-*dimethyl*-6'-*methylene*-3'-*tetrahydropyranyloxycyclohexyl*)-3-*methyl*-2-*penten*-4-*yn*-1-*yl*-*tetrahydroopyranyl ether* (**8a** *and* **9a**). EtBr (14.0 g) in dry THF (25 l) was added dropwise to a suspension of Mg dust (2.46 g) in dry THF (50 ml) with stirring at 25–30°. After 20 min, (2*Z*)-3-methyl-2-penten-4-yn-1-yl THP ether (18.4 g) in dry THF (20 ml) was added within 30 min at 0–5° and stirred for 30 min at room temp. To this sol was added (*S*)-**7a** (8.8 g) in dry THF (10 ml) at 0–10°. After stirring overnight at room temp., the mixt. was poured into a cold satd NH_4Cl soln and extracted with Et_2O. Following evap of the organic solubles, the residual oil was chromatographed over silica (400 g). Elution with *n*-hexane–EtOAc (10:1) gave (2*Z*)-3-methyl-2-penten-4-yn-1-yl THP ether (7.8 g). Further elution with *n*-hexane–EtOAc (7:1 and 3:1) gave **8a** (7.12 g, 46% yield) and **9a** (4.32 g, 28% yield, as a mixture of two isomers), respectively.

(+)-(1'S, 3'S)-(Z)-5-(1', 3'-*Dihydroxy*-2', 2'-*dimethyl*-6'-*methylenecyclohexyl*)-3-*methyl*-2-*penten*-4-*yn*-1-*ol* (**8**). HOAc (100 ml) was added to a soln of **8a** (6.00 g) in THF (50 ml) and H_2O (50 ml). After stirring for 2 days at room temp., the mixt. was poured into cold H_2O, neutralized

with K_2CO_3 powder (115 g) and extracted with EtOAc. Following evap. of the organic solubles *in vacuo*, the residual oil was chromatographed over silica (50 g). Elution with *n*-hexane–EtOAc (1:2) gave **8** (3.90 g, 92% yield) as a solid. Recrystallization from Et_2O gave crystalline needles, mp 45–46°. $[\alpha]_D^{20}$ + 19.0° ($CHCl_3$, *c* 1.73). IR ν_{max}^{film} cm^{-1}: 3350, 3080, 1705, 1645, 1640, 1440, 1010. EIMS (GC) 70 eV, *m/z* (rel. int.): 232 $[M - H_2O]^+$ (30), 214 (42), 199 (33), 186 (32), 176 (82), 175 (44), 171 (36), 161 (100), 153 (59), 123 (35); ^{1}H NMR (100 MHz, $CDCl_3$): δ0.98 (3H, *s*), 1.24 (3H, *s*), 1.90 (3H, *d*, *J* = 1.6 Hz), 1.50–2.68 (4H, *m*), 3.64 (1H, *br.s*), 4.29 (2H, *d*, *J* = 6.8 Hz), 4.97 (1H, *s*), 5.34 (1H, *s*), 5.88 (1H, *td*, *J* = 6, 8 and 1.6 Hz). Found: C, 71.54; H, 8.95. $C_{15}H_{22}O_3$ requires: C, 71.97; H, 8.86%.

(+)-(1′R, 3′S)-(2Z, 4E)-5-(1′, 3′-*Dihydroxy*-2′,2′-*dimethyl*-6′-*methylenecyclohexyl*)-3-*methyl*-2,4-*pentadien*-1-*ol* (**10**). To a soln of Redal (70% soln in toluene, 10 g) in dry THF (40 ml), **8** (3.90 g) was added dropwise with stirring at 0°. After stirring overnight at room temp., the mixt. was poured into a cold satd NH_4Cl soln, and the salt generated was filtered off. The filter cake and the filtrate were extracted with EtOAc. Following, evap of the organic solubles *in vacuo*, the residual oil was chromatographed over silica (30 g). Elution with *n*-hexane–EtOAc (1:2) gave **10** (3.02 g, 77% yield) as a semi-solid.

Recrystallization was not successful. $[\alpha]_D^{20}$ + 37.4° ($CHCl_3$, *c* 0.503). UV λ_{max}^{EtOH} nm (log ε): 238.0 (4.15). IR ν_{max}^{film} cm^{-1}: 3340, 3080, 1640, 1615. EIMS (GC) 70 eV, *m/z* (rel. int.): 234 $[M - H_2O]^+$ (30), 214 (42), 201 (92), 173 (60), 161 (55), 107 (100). ^{1}H NMR (100 MHz, $CDCl_3$): δ0.89 (3H, *s*), 1.01 (3H, *s*), 1.90 (3H, *d*, *J* = 1.60 Hz), 1.52–2.77 (4H, *m*), 3.56 (1H, *br.s*), 4.28 (2H, *d*, *J* = 7.2 Hz), 4.81 (1H, *s*), 4.92 (1H, *s*), 5.56 (1H, *d*, *J* = 7.2 Hz), 5.56 (1H, *d*, *J* = 15.6 Hz), 6.02 (1H, *d*, *J* = 15.6 Hz).

(+)-(1′R, 3′S)-*Methyl*-1′, 3′-*dihydroxy*-γ-*ionylideneacetate* (**11a**). Activated MnO_2 (30 g) was added to a soln of **10** (3.02 g) in dry CH_2Cl_2 (250 ml), and the mixt. was shaken at room temp for 2 hr. The MnO_2 was filtered off and washed with EtOAc. The filtrate was evapd to give the crude aldehyde, which was then added to a mixture of activated MnO_2 (30 g), powdered NaCN (3.0 g) and HOAc (3.0 g) in MeOH (250 ml). The mixt. was shaken overnight at room temp. After removal of the MnO_2 by filtration, the filtrate was concd, extracted with EtOAc and the results organic solubles evaporated *in vacuo*. The residual oil was chromatographed over silica (40 g). Elution with *n*-hexane–EtOAc (1:1) gave **11a** (2.65 g, 79% yield) as a white crystal. Recrystallization from hexane–EtOAc (1:1) gave crystalline needles, mp 119–200°, $[\alpha]_D^{20}$ + 77.9 ($CHCl_3$, *c* 0.296). CD: $[\Phi]_{246}$ 0, $[\Phi]_{250}$ + 1.5 × 10^4, $[\Phi]_{260}$ + 7.6 × 10^4, $[\Phi]_{265}$ + 8.5 × 10^4, $[\Phi]_{270}$ + 6.4 × 10^4, $[\Phi]_{280}$ + 5.4 × 10^4, $[\Phi]_{290}$ + 2.2 × 10^4 (MeOH, *c* 0.111). UV λ_{max}^{EtOH} nm (log ε): 265.1 (4.29). IR ν_{max}^{KBr} cm^{-1}: 3360, 3080, 1710, 1640, 1600, 1435, 1360, 1265, 1230. EIMS (GC) 70 eV, *m/z* (rel. int.): 280 $[M]^+$ (1), 279 (2), 262 $[M - H_2O]^+$ (51), 244 (57), 197 (55), 171 (100), 128 (52). ^{1}H NMR (100 MHz, $CDCl_3$): δ0.95 (3H, *s*), 1.06 (3H, *s*), 2.05 (3H, *d*, *J* = 1.0 Hz), 1.69–2.73 (4H, *m*), 3.67 (1H, *br. s*), 3.71 (3H, *s*), 4.84 (1H, *s*), 4.97 (1H, *s*), 5.73 (1H, *d*, *J* = 1.0 Hz), 6.38 (1H, *d*, *J* = 15.7 Hz), 7.85 (1H, *d*, *J* = 15.7 Hz). Found: C, 68.54; H, 8.47. $C_{16}H_{24}O_4$ requires: C, 68.54; H, 8.63%. These authentic data were identical with those for authentic material from *C. cruenta* [11].

(+)-(1′R, 3′*S*)-1′, 3′-*Dihydroxy*-γ-*ionylideneacetic acid*, [(1′R, 3′*S*)-(2*Z*, 4*E*)-5-(1′, 3′-*dihydroxy*-2′, 2′-*dimethyl*-6-*methylenecyclohexyl*)-3-*methyl*-2, 4-*pentadienoic acid*] (**11**). To a soln of **11a** (200 mg) in MeOH (3.0 ml), 20% aq. KOH (0.4 ml) was added at 0°C. After stirring overnight at room temp., the mixture was poured into cold H_2O and extracted with Et_2O. The aq. layer was acidified to pH 3 with 20% H_3PO_4 soln. extracted with EtOAc. The EtOAc solubles were combined and evaporated *in vacuo*. residual oil was purified by prep. resulting TLC, eluted with C_6H_6–EtOAc–HOAc (60:40:1). Obtained semi-solid was recrystallized from EtOAc to give pure **11** (171 mg, 86% yield) as crystalline needles mp 141–145°, $[\alpha]_D^{20}$ + 29.9° ($CHCl_3$, *c* 0.850). UV λ_{max}^{EtOH} nm (log ε): 261 (4.28). IR ν_{max}^{KBr} cm^{-1}: 3400, 3080, 1710, 1640, 1600, 1420, 1360, 1220. EIMS (GC): 70 eV, *m/z* (rel. int.) 248 $[M - H_2O]^+$ (26), 230 (74), 215 (44), 204 (86), 197 (47), 171 (96), 159 (97), 145 (88), 105 (100), 91 (92). ^{1}H NMR (270 MHz, $CDCl_3$): δ0.92 (3H, *s*), 1.05 (3H, *s*), 2.07 (3H, *s*), 1.77–2.91 (4H, *m*), 3.58 (1H, *br.s*), 4.80 (1H, *s*), 4.96 (1H, *s*), 5.73 (1H, *s*), 6.39 (1H, *d*, *J* = 16.1 Hz), 7.81 (1H, *d*, *J* = 16.1 Hz). Found: C, 67.16; H, 8.25. $C_{15}H_{22}O_4$ requires: C, 67.64; H, 8.33%.

(+)-(R)-*Methyl*-1′-*hydroxy*-3′-*oxo*-γ-*ionylideneacetate* (**2a**). To a stirred soln of **11a** (200 mg) in dry CH_2Cl_2 (10 ml), PCC (308 mg) was added. After stirring for 3 hr at room temp., the mixt. was filtered through silica column. The eluate was concd and purified by prep. TLC with hexane–EtOAc (4:1) to give **2a** (142 mg, 71%) as a yellow solid, mp 129–130°, $[\alpha]_D^{20}$ + 5.9° ($CHCl_3$, *c* 0.290). UV λ_{max}^{EtOH} nm (log ε): 265.8 (4.35); IR ν_{max}^{KBr} cm^{-1}: 3080, 1710, 1640, 1600, 1265, 1235, 1160; EIMS (GC) 70 eV, *m/z* (rel. int.): 278 $[M]^+$ (19), 260 $[M - H_2O]^+$ (58), 246 (23), 228 (35), 219 (79), 175 (55), 173 (57), 125 (100); ^{1}H NMR (100 MHz, $CDCl_3$): δ1.11 (6H, *s*), 2.01 (3H, *d*, *J* = 1.2 Hz), 2.41–2.78 (4H, *m*), 3.70 (3H, *s*), 5.12 (1H, *s*), 5.19 (1H, *s*), 5.73 (1H, *d*, *J* = 1.2 Hz), 6.27 (1H, *d*, *J* = 16.5 Hz), 7.84 (1H, *d*, *J* = 16.5 Hz). Found: C, 69.24; H, 8.01. $C_{16}H_{22}O_4$ requires: C, 69.04; H, 7.97%.

(+)-(R)-1′-*Hydroxy*-3′-*oxo*-γ-*ionylideneacetic acid* (**2**). To a stirred soln of **2a** (200 mg) in dry THF (10 ml), Dess–Martin periodinate [18] (380 mg) was added. After stirring for 3 hr at room temp., the mixt. was filtered through a silica column. The eluate was concd and purified by prep. TLC with *n*-hexane–EtOAc–HOAc (60:4:1) to give **2** (188 mg, 95%) as a solid, mp 117–118°, $[\alpha]_D^{20}$ + 7.8° ($CHCl_3$, *c* 1.31). UV λ_{max}^{EtOH} nm (log ε): 259.2 (4.25). IR ν_{max}^{KBr} cm^{-1}: 3500, 3250, 3080, 1690, 1680, 1630, 1600, 1460, 1440, 1270, 1250, 1120, 1110. EIMS (GC) 70 eV, *m/z* (rel. int.): 264 $[M]^+$ (33), 246 $[M - H_2O]^+$ (19), 231 (7), 221 (100), 203 (16), 193 (49), 111 (80), 97 (100). ^{1}H NMR (270 MHz, $CDCl_3$): δ1.09 (3H, *s*), 1.11 (3H, *s*), 2.04 (3H, *s*), 2.27–2.86 (4H, *m*), 5.12 (1H, *s*), 5.76 (1H, *s*), 6.32 (1H, *d*, *J* = 15.9 Hz), 7.81 (1H, *d*,

J = 15.9 Hz). HPLC (25°, 5.0 ml min^{-1}): 10.1 min (100%). Found: C, 68.10; H, 7.54. $C_{15}H_{20}O_4$ requires: C, 68.16; H, 7.63%.

Treatment of **11** *by PCC (formation of* **12**). To a stirred soln of **11** (75 mg) in dry CH_2Cl_2 (10 ml) was added PCC (116 mg). After stirring for 3 hr at room temp., the mixt. was filtered through silica column. The elute was concd and purified by prep. TLC with *n*-hexane–EtOAc–HOAc (70:30:1) to give **12** (21 mg, 29%) as an oil. IR ν_{max}^{KBr} cm^{-1}: 3080, 1760, 1720, 1640, 960. EIMS (GC) 70 eV, *m/z* (rel. int.): 246 $[M]^+$ (7), 231 (3), 202 (4), 159 (6), 149 (100), 121 (11), 107 (16), 91 (17). ^{1}H NMR (270 MHz, $CDCl_3$): δ1.25 (3H, *s*), 1.27 (3H, *s*), 1.98 (3H, *s*), 2.43–2.74 (4H, *m*), 5.07 (1H, *d*, J = 9.5 Hz), 5.15 (1H, *s*), 5.36 (1H, *s*), 5.64 (1H, *d*, J = 9.5 Hz), 5.85 (1H, *s*). Found: C, 732.66; H, 6.85. $C_{15}H_{18}O_3$ requires: C, 73.15; H, 7.36%.

Treatment of **2** *by acidic catalysts.* To a stirred soln of **2** (10 mg) in MeOH (1.0 ml), an acidic catalyst (10.0 mol%) was added, and the reaction was monitored by HPLC (260 nm). When H_2SO_4, H_3PO_4, HCl, HOAc, TFA or camphorusulphonic acid was employed as the catalyst, the single peak of **2** disappeared within 4 hr at room temp. and no other peak was generated. The disappearance of **2** was more sluggish when $ZnCl_2$, $RhCl_2$ or pyridinium *p*-toluenesulphonate was used. No breakdown fragment or any other product from **2** was recovered in every case.

(−)-(1′R, 4′R, 6′R)-(Z)-5-(1′, 4′-*Dihydroxy*-2′, 2′, 6′-*trimethylcyclohexyl*)-3-*methyl*-2-*penten*-4-*yn*-1-*ol* (**15**). To a soln of (2*Z*)-3-methyl-2-penten-4-yn-1-yl THP ether (9.00 g) in dry THF (100 ml), *n*-BuLi (2.5 M soln in *n*-hexane, 48 ml) was added at 0°. After stirring for 30 min at room temp., (4*R*, 6*R*)-**14a** (9.00 g, 99% e.e.) [19] was added within 1 hr at room temp. and further stirred for 3 hr. The mixt. was poured into a cold satd NH_4Cl soln and extracted with Et_2O. Crude **15a** was then dissolved in 15% TFA soln in MeOH (100 ml) and stirred for 4 hr at room temp. The residual oil, following evap of the organic solvent *in vacuo*, was purified by flash chromatography with *n*-hexane–EtOAc (2:1) to give **15** (8.03 g, 85% yield) as a solid. Recrystallization from Et_2O gave crystalline needles, mp 163–165°, $[\alpha]_D^{20}$ − 24.2° (EtOH, *c* 2.44). IR ν_{max}^{KBr} cm^{-1}: 3350, 1640, 1060, 1040, 1020. EIMS (GC) 70 eV *m/z* (rel. int.): 252 $[M]^+$ (1), 234 $[M - H_2O]^+$ (13), 216 (4), 203 (4), 201 (5), 178 (15), 165 (18), 148 (100), 121 (43), 106 (58). ^{1}H NMR (270 MHz, $CDCl_3$): δ1.09 (3H, *d*, J = 6.6 Hz), 1.11 (3H, *s*), 1.25 (3H, *s*), 1.90 (3H, *s*), 1.57–2.40 (5H, *m*), 4.05 (1H, *br.s*), 4.43 (2H, *d*, J = 6.5 Hz), 5.87 (1H, *t*, J = 6.5 Hz). Found: C, 71.23; H, 9.70. $C_{15}H_{24}O_3$ requires: C, 71.39; H, 9.59%.

(−)-(1′S, 4′R,6′R)-(2Z, 4E)-5-(1′, 4′-*Dihydroxy*-2′, 2′, 6′-*trimethylcyclohexyl*)-3-*methyl*-2, 4-*pentadien*-1-*ol* (**16**). To a soln of Redal (20 ml of 65% soln in toluene, Aldrich) in dry THF (75 ml), **15** (4.88 g) was added dropwise with stirring at 0°. After stirring overnight at room temp., the mixt. was quenched by adding H_2O with ice-bath cooling, and the salt generated was filtered off. The filter cake was washed by EtOAc. The extract was concd, and the residual oil was purified by flash chromatography with *n*-hexane–EtOAc (2:1) to give **16** (3.22 g, 66% yield) as a white solid.

Recrystallization from Et_2O gave crystal, line needles, mp 74–76°, $[\alpha]_D^{20}$ − 32.8° (EtOH, *c* 1.24). IR ν_{max}^{KBr} cm^{-1}: 3350, 1620, 1040, 1000, 960. EIMS (GC) 70 eV, *m/z* (rel. int.): 254 $[M]^+$ (5), 236 $[M - H_2O]^+$ (40), 218 (16), 168 (100), 150 (51), 135 (67), 124 (71), 109 (91). ^{1}H NMR (270 MHz, $CDCl_3$): δ0.81 (3H, *s*), 0.81 (3H, *d*, J = 8.9 Hz), 1.25 (3H, *s*), 1.87 (3H, *d*, J = 1.0 Hz), 1.27–2.37 (5H, *m*), 4.12 (2H, *br.t*, J = 6.1 Hz), 4.35 (1H, *d*, J = 7.3 Hz), 5.56 (1H, *dt*, J = 1.0, 7.3 Hz), 5.89 (1H, *d*, J = 15.6 Hz), 6.72 (1H, *d*, J = 15.6 Hz). Found. C, 71.10; H, 10.34. $C_{15}H_{26}O_3$ requires: C, 70.83; H, 10.30%.

(−)-(1′R, 2′R)-2′, 3′-*Dihydroabscisic acid* (**5**). In the same manner as **10**, **16** (1.20 g) was converted into the methyl ester, which, following alkaline hydrolysis and Dess–Martin oxidation, gave **5** (0.60 g, 48% overall yield). Recrystallization from MeCN gave a needle crystal, mp 170–172°, $[\alpha]_D^{20}$ − 69.5° (MeOH, *c* 0.364). IR ν_{max}^{KBr} cm^{-1}: 3460, 3210, 1700, 1680, 1660, 1630, 1600, 1300, 1280, 1250. EIMS (GC as **5a**) 70 eV, *m/z* (rel. int.): 280 $[M]^+$ (15), 262 $[M - H_2O]^+$ (7), 248 (6), 224 (12), 196 (25), 192 (76), 164 (29), 123 (100). ^{1}H NMR (270 MHz, d_4-MeOH): δ0.90 (3H, *d*, J = 5.9 Hz), 0.93 (3H, *s*), 1.01 (3H, *s*), 2.10 (3H, *d*, J = 1.0 Hz), 2.03–2.05 (4H, *m*), 2.72 (1H, *d*, J = 14.6 Hz), 5.73 (1H, *s*), 6.61 (1H, *d*, J = 15.8 Hz), 7.89 (1H, J = 15.8 Hz). HPLC (25°, 5.0 ml min^{-1}): 10.6 min (100%) (*cf.* ABA: 10.8 min). Found: C, 67.65; H, 8.31. $C_{15}H_{22}O_4$ requires: C, 67.65; H, 8.32%.

(1′S, 2′S)-2′, 3′-*Dihydroabscisic acid* (**5**). In the same manner as the synthesis of (−)-**5**, (4*R*, 6*S*)-**17a** (3.21 g, 99% e.e.) [19] was converted into (+)-**5** (0.75 g, 21% overall yield), mp 170–172°, $[\alpha]_D^{20}$ + 70.6° (MeOH, *c* 0.279). Spectral data for both enantiomers of **5** were almost identical with those reported previously [10, 16].

Stomatal closure assay. The epidermis for the biological activity assay was isolated from *C. communis*. Samples were dissolved in 1.0 ml citrate buffer (pH 5.6). Synthetic (*S*)-ABA [25] was used as standard. Apertures of stomata were measured under a microscope after incubation for 3 hr at 27° under illumination.

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