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ACHIRAL CYCLOHEXADIENONE ANALOGUES OF ABSCISIC ACID: SYNTHESIS AND BIOLOGICAL ACTIVITY*

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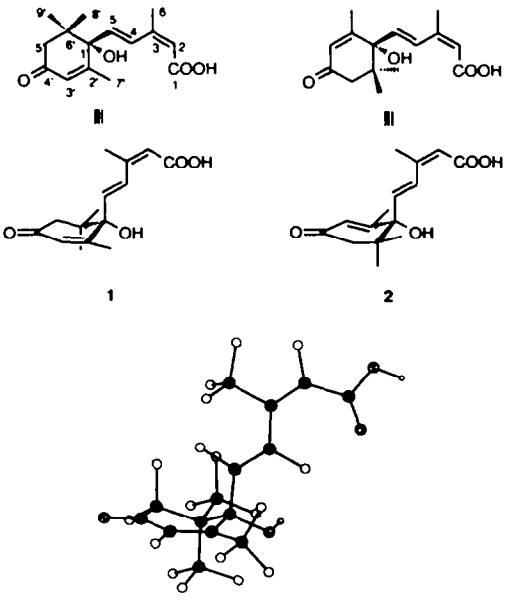
Key Word Index—*Bromus inermis*; Gramineae; *Lepidium sativum*; Cruciferae; freezing tolerance; germination; synthesis; abscisic acid; ABA analogues.

Abstract—Novel achiral cyclohexadienone (divinyl) analogues of abscisic acid (ABA) were synthesized and tested for biological activity. Oxidation of 2,6-dimethylphenol with iodobenzene acetate in ethylene glycol and hexane afforded 2,6-dimethyl-4,4-ethylenedioxycyclohexa-2,5-dienone as the key intermediate which was further transformed to a series of ABA analogues with cyclohexa-2',5'-dien-4'-one ring and dienoic or enynoic sidechains at different oxidation levels. Their biological activity was compared to racemic ABA in assays for germination of cress seeds and freezing tolerance in bromegrass cell suspension cultures and related to the activity of the corresponding analogues with the ABA ring system. The divinyl dienoic methyl ester was as active as racemic ABA and methyl ABA in inhibiting cress seed germination. The divinyl dienoic methyl ester and divinyl acetylenic methyl ester had comparable activity to methyl ABA in inducing freezing tolerance. The results suggest that the vinyl methyl group is sufficient for recognition by ABA response systems in these assays and the axial methyl is not absolutely required.

INTRODUCTION

Recent studies with optically pure enantiomers of the plant hormone (*S*)-(+)-abscisic acid [(*S*)-(+)-ABA, **1**] indicate that the stereochemical requirements for ABA perception vary with different species, developmental stages and physiological processes. Natural ABA **1** is a strong inhibitor of cress seed germination (*Lepidium sativum*) whereas the unnatural analogue (*R*)-(−)-abscisic acid (**2**) is only weakly active [1]. In contrast, both **1** and **2** are equally effective inhibitors of germination of excised wheat embryos [2]. The same stereochemical discrimination in response is observed in stomatal opening [3] and in induction of mRNA coding for storage proteins in microspore-derived embryos of *Brassica napus* [4], and in freezing tolerance in cultured bromegrass cells [5].

In Fig. 1, molecules **1** and **2** are shown in the conformations determined by X-ray crystallography [6, 7] and solution state NMR spectroscopic studies [8]. In both structures the hydroxyl group is equatorial and the sidechain is axial. The difference between **1** and **2** lies in the position of the ring methyl groups. If one imagines a plane bisecting the ring from C-4' to C-1' and including the hydroxyl and sidechain, in **1** the vinyl methyl is in front of the plane and the geminal dimethyl behind, one methyl group axial and the other equatorial. In unnatural ABA **2** the geminal dimethyl group is in front and the vinyl methyl behind. The role of the axial methyl group of either ABA molecule has not been established in assays in which a differential response is observed.



1 2

Computer-generated structure of 1 from X-ray data [6,7]

Fig. 1.

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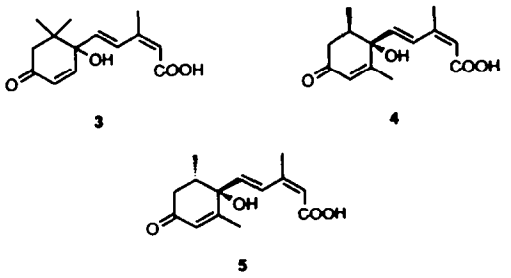
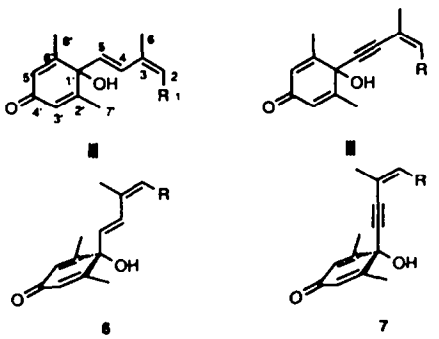


Fig. 2.



R = COOH, $COOCH_3$, CHO, CH_2OH

Fig. 3.

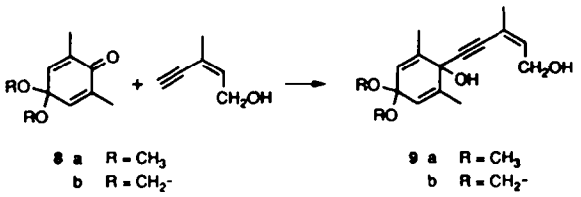
Nanzyo *et al.* [9] synthesized the racemic ABA analogue **3**, lacking the C-7′ methyl group, as well as the racemic diastereomers **4** and **5**, lacking either the C-8′ or C-9′ methyl group. They found that racemates **4** and **5** inhibited rice seedling growth while **3** did not, and concluded that the C-7′ methyl was required for activity. However, they did not address the requirement of the axial methyl group in ABA recognition.

We propose that ABA analogues **6** and **7** (Fig. 3), containing two ring vinyl methyl groups, would allow us to probe the requirement of the axial methyl group. In addition there is no question of the effect of chirality in these analogues, as the molecules have a plane of symmetry and therefore are achiral. The predicted 3D structures of these divinyl analogues are shown in Fig. 3. We report here the synthesis of novel ABA analogues incorporating the divinyl ring system with both dienoic and enynoic sidechain, and the preliminary assessments of their biological activity in inhibiting cress seed germination and in increasing the freezing tolerance of bromegrass cells grown in culture.

RESULTS AND DISCUSSION

Synthesis

Our strategy for obtaining achiral cyclohexadienone (divinyl) ABA analogues was based on a practical synthesis of ABA described by Mayer [10], that has been successfully employed in our laboratory for ABA ana-



Scheme 1.

logues and metabolites [11-13]. The key intermediate in the synthesis is 2,6-dimethyl-1,4-benzoquinone with the C-4 protected as an acetal. The alkylation product would be suitable for conversion into a number of derivatives, either enynoic or dienoic and with different oxidation levels of the terminal carbon of the sidechain (Scheme 1).

A novel method for synthesizing the required cyclohexadienone monoketal was developed. Initial efforts to ketalize directly 2,6-dimethyl-1,4-benzoquinone were unsuccessful. A procedure [14] to oxidize 2,6-dimethylphenol with iodobenzene acetate in methanol only gave a low yield of the dimethyl acetal **8a**. However, the latter reaction looked more promising and two modifications were made; the methanol was replaced with ethylene glycol and hexane was added to make a biphasic solution. With these alterations the reaction proceeded to completion and the product was readily purified from another byproduct to give pure cyclohexadienone **8b** in 63% yield. The general applicability of this reaction as a route to preparing a variety of 4-oxoprotected cyclohexadienones is currently under investigation.

The sidechain was then introduced into the ring system at C-1′ position by alkylation of the dianion of *cis*-3-methyl-2-penten-4-yn-ol to afford the diol derivative **9b** in 81% yield (Scheme 2).

The transformations to acetylenic analogues were straightforward. HCl (10%) in acetone was used to remove the ketal protecting group of **9b** to give ketoalcohol **10** in 87% yield, this being subsequently converted via manganese dioxide oxidation to ketoaldehyde **11** in 72% yield. Methyl ester **12** was obtained in 84% yield by Corey oxidation [15] of aldehyde **11**. All attempts to convert the ester **12** to the acid were unsuccessful due to instability of the product.

Analogues with a dienoic sidechain were prepared from the ketal alcohol **9b** by reduction to intermediate **13** using sodium bis(2-methoxyethoxy)aluminium hydride (Redal®) at -78° in THF. The reduction product **13** was not stable to silica gel chromatography and was transformed directly into the aldehyde **16** and methyl ester **18** by methods similar to those used for making the acetylenic analogues. Alcohol **13** was converted to aldehyde **15** using manganese dioxide in acetone and the crude product was used directly in a Corey oxidation to form methyl ester **17** (*ca* 25% from **9b**). Compound **17** proved to be stable and was purified by silica gel chromatography.

The ketal protecting groups were removed from alcohol **13**, aldehyde **15** and methyl ester **17** by reaction with 10% HCl in acetone (yields 80-90%), to give the ketoalcohol **14**, ketoaldehyde **16** and methyl ester **18** respect-

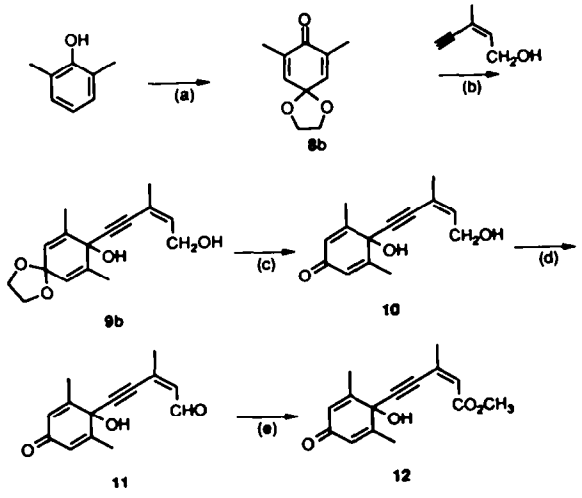
ively. Basic hydrolysis of ketal-ester **17** using 2 M KOH in methanol was successful and following acidification afforded acid **19** in 63% yield.

The 3D drawings of **12** and **18** in Fig. 4 represent their low energy structure conformations which are built with SYBYL [16] software using the POWELL minimization method and the Tripos force field. The computer-generated structures show the cyclohexadienonyl rings in these molecules are planar. The ring vinyl methyl of **12**, **18** and ABA have similar spatial requirements.

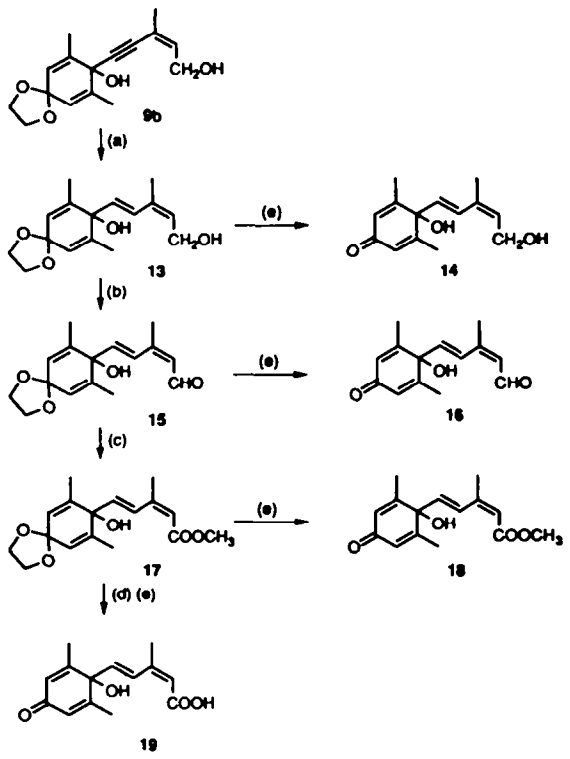
Biological results

Cress seed germination. The activity of new divinyl analogues was assayed and compared to (±)-ABA in inhibiting the germination of cress seed, a system previously used to compare the activity of ABA, its enantiomers and racemic forms of ABA metabolites [1]. Abscisyl alcohol and its corresponding aldehyde were more potent than (*S*)-ABA in inhibiting root and leaf emergence, while unnatural (*R*)-ABA displayed only weak activity.

Comparison of racemic methyl abscisate **20** and the methyl ester of the acetylenic analogue **21** (Fig. 5) with their corresponding divinyl analogues was carried out so that the ring systems were the only variables. The ABA analogues **20** and **21** were more active than racemic ABA in inhibition root emergence (Fig. 6). At a concentration of 100 μM, both esters inhibited root emergence for more than 48 hr. The potent inhibitory effect of methyl ABA has previously been attributed to a combination of several factors including more facile transport and slow metabolism to ABA, thus providing a longer pulse of



Scheme 2. (a) $C_6H_3I(OAc)_2$, $HOCH_2CH_2OH$–hexane; (b) 2 eq. *n*-BuLi–THF, −78°; (c) 10% HCl–acetone; (d) MnO_2–acetone; (e) MnO_2–NaCN–MeOH.



Scheme 3. (a) Red-Al®–THF; (b) MnO_2–acetone; (c) MnO_2–NaCN–HOAc–MeOH; (d) 2 M KOH–MeOH; (e) 10% HCl–acetone.

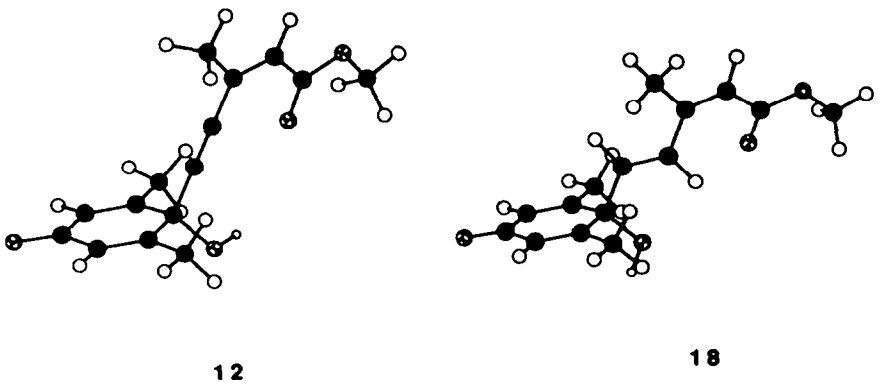


Fig. 4.

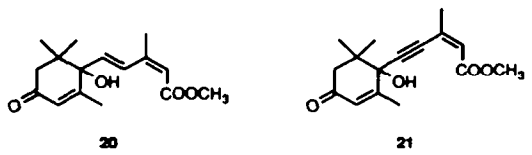


Fig. 5.

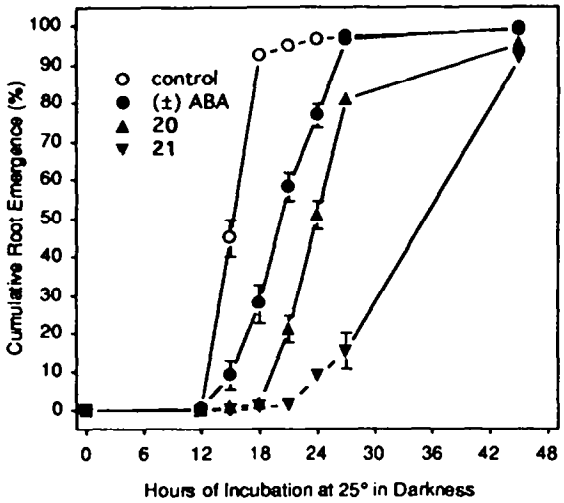


Fig. 6. The effect of (±)-ABA, **20** and **21** (10 μM) on root emergence of cress seeds.

ABA [17]. In a wheat embryo germination assay, both optically active forms of methyl ABA and methyl acetylenic ABA displayed similar activities [4]. Acetylenic ABA analogues show activity in ABA assays [18].

Next, the activity of the divinyl methyl ester **18** and acid **19** were compared to racemic ABA (all at 10 μM) for inhibitory effects on root emergence of cress seeds (Fig. 7). The dienoic acid **19** was inactive, likely due to instability of the compound in solution. (In the preparation of **19**, the compound was very sensitive to dilute base.) The dienoic methyl ester **18** had similar activity to (±)-ABA, paralleling the observed activities of methyl ABA **20** and acetylenic ABA methyl ester **21**. This result supports the hypothesis that the ABA perception system in cress seeds recognizes the analogue containing only the vinyl methyl group and does not require the axial methyl.

The acetylenic divinyl methyl ester **12** was active in germination inhibition (Fig. 8), but was relatively weaker than (±)-ABA. It is not known if the weak activity of **12** is due to instability of the compound or if it is poorly recognized by the ABA perception system.

In the ABA series, the aldehyde and alcohol were more active than the acid whereas in the divinyl series the alcohols **10** and **14** are inactive and the aldehydes **11** and **16** are weakly active (not shown). Oxidation at C-1 in the divinyl series may be blocked, or alternatively perhaps the abscisyl alcohol is itself active but the divinyl alcohols are not.

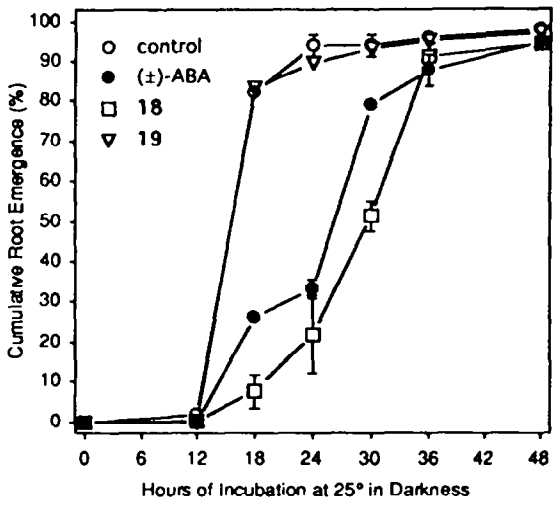


Fig. 7. The effect of (±)-ABA, **18** and **19** (10 μM) on root emergency of cress seeds.

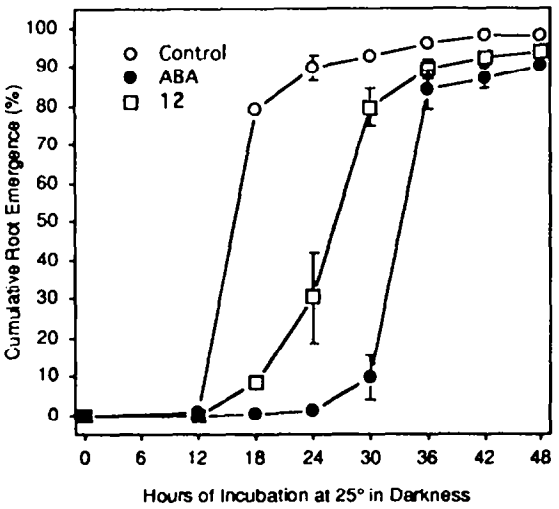


Fig. 8. The effect of (±)-ABA, and **12** (10 μM) on root emergence of cress seeds.

Cold hardening in bromegrass cells. Cultured bromegrass cells cold harden in response to exogenous ABA at nonhardening temperatures (25°) [19]. In a previous study, racemic ABA analogues were compared to ABA in inducing cold hardening [20]. The abscisyl alcohol, abscisyl aldehyde and acetylenic ABA were inactive. Both methyl ABA and methyl acetylenic ABA had comparable intermediate activity. In the present case, divinyl ABA analogues were compared to racemic ABA in inducing freezing tolerance at 100 μM in the bromegrass assay. The acetylenic series and the dienoic series were conducted in two separate experiments (Figs 9 and 10). The divinyl acetylenic aldehyde **11** made the cells more sensitive to freezing, probably due to toxicity of the compound at 100 μM. The other compounds **10**, **14**, **16** and **19** were inactive in inducing freezing tolerance. The divinyl dienoic methyl ester **18** was active with an $LT_{50} = -20.7°$

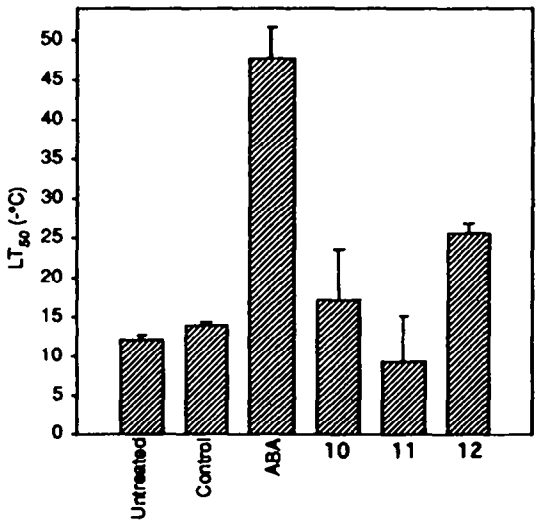


Fig. 9. The effect of (±)-ABA, **10**, **11** and **12** (100 μM) on the freezing tolerance of bromegrass suspension cells after seven days at 25°. *LT_{50} = 50% killing temperature determined by triphenyltetrazolium chloride (TTC) reduction viability assay.

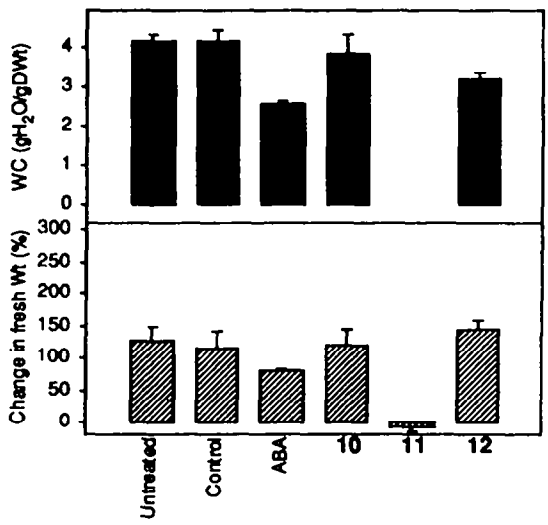


Fig. 11. The effect of (±)-ABA, **10**, **11** and **12** (100 μM) on water content (WC) and growth of bromegrass cells after seven days at 25°. *Not determined.

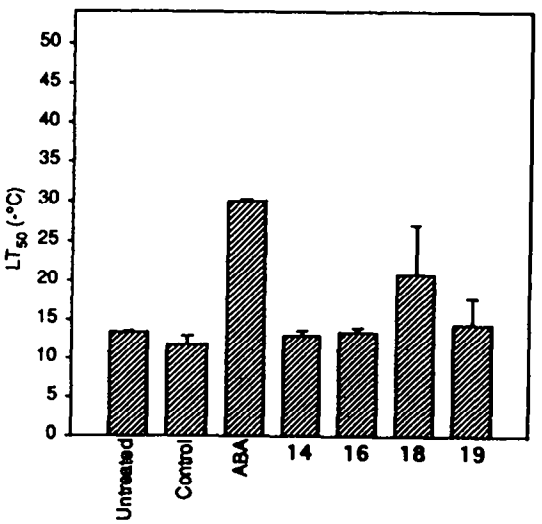


Fig. 10. The effect of (±)-ABA, **14**, **16**, **18** and **12** (100 μM) on the freezing tolerance of bromegrass suspension cells after seven days at 25°. *LT_{50} = 50% killing temperature determined by TTC reduction viability assay.

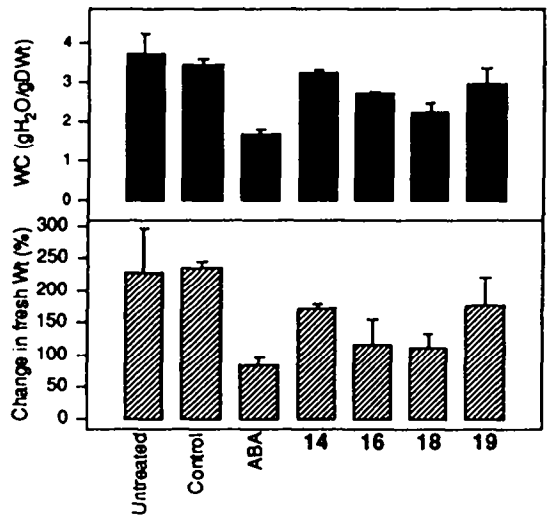


Fig. 12. The effect of (±)-ABA, **14**, **16**, **18** and **19** (100 μM) on water content (WC) and growth of bromegrass cells after seven days at 25°.

compared to −30° for (±)-ABA and −13.2° for the control. The divinyl acetylenic methyl ester **12** also had similar intermediate activity with an LT_{50} = −25.5° compared to −47.9° for (±)-ABA and −12.2° for the control.

Both growth characteristics and water content of cells were reduced in the acclimation process. These effects were examined using divinyl ABA analogues and compared to racemic ABA (all at 100 μM concentration) in the bromegrass assay (Figs 11 and 12). The divinyl analogues caused a slight reduction of water content of the cells. The divinyl dienoic compounds reduced growth compared to the control, while the two acetylenic divinyl compounds did not. The divinyl acetylenic aldehyde **11** resulted in a negative growth and no data for water content were obtained.

These results demonstrate that achiral cyclohexadienone analogues of abscisic acid are perceived as ABA-like in both assays examined. The methyl esters **12** and **18** are active in inhibiting root emergence of cress seeds and are moderately active in inducing freezing tolerance of bromegrass cells. It is concluded that the vinyl methyl system is sufficient for recognition, and that the presence of the axial methyl group is not critical for activity in cress

seed germination and freezing tolerance in bromegrass cells.

EXPERIMENTAL

General. Mps are uncorr. IR spectra were recorded using $CHCl_3$ as solvent and matching NaCl cavity cells (0.2 mm). Proton nuclear magnetic resonance (^{1}H NMR) was recorded at 360 or 500 MHz. Carbon-13 (^{13}C NMR) spectra were recorded at 90 or 125 MHz. Unless otherwise stated, $CDCl_3$ was used as solvent in all NMR experiments with $CHCl_3$ as ref. Chemical shifts (δ) and coupling constants (J) are reported as if they are first order. Low resolution mass spectra (LCMS) were obtained by using a DB-5 column (60 m) in a Finnigan-Mat 4000 E instrument with an Incos 2300 data system operated in either the electron impact (EI) mode or the chemical ionization (CI) mode. Mass spectral data are reported in mass to charge units (m/z). High resolution mass spectra (HRMS) were recorded in the electron impact mode using VG 70-250SEQ double-focusing hybrid spectrometer with a Digital PDP 11/73 data system.

Flash CC was performed using Merck silica gel 60 (230–400 mesh). Merck silica gel 60 F254 plates (0.2 mm) with aluminium sheet backing was used in analytical TLC. UV active materials were detected under an UV lamp. The plates were then dipped into a soln of phosphomolybdic acid and heated on a hot plate to visualize the spots.

The solvent tetrahydrofuran (THF) was dried by distillation from sodium and benzophenone. Unless otherwise indicated, all reactions were conducted under the atm. of dry argon.

2,6-*Dimethyl*-4,4-*ethylenedioxycyclohexa*-2,5-*dienone* **(8b)**. Iodobenzene diacetate (20.29 g, 0.063 mol) was suspended in hexane (250 ml), mechanically stirred, and cooled by means of an external ice bath. To the suspension was added 2,6-dimethylphenol (3.66 g, 0.030 mol) in ethylene glycol (20 ml). The mixt. was allowed to warm to room temp., stirred for 2 hr, and then quenched with H_2O (200 ml). The phases were sepd and the aq. layer was extracted with ether (200 ml). The combined organic layers were washed with 5% $NaHCO_3$ and satd NaCl soln, dried ($MgSO_4$) and concd. Distillation *in vacuo* removed most iodobenzene and the residue was purified by flash chromatography (EtOAc–hexane 1:20) to give 3.38 g (63%) of **8b** which was further purified by recrystallization (ether–hexane) to give a crystal. Mp: 47–49°, IR λ_{max}^{MeCl} cm^{-1}: 1715 (C=O), 1630 (C=C); ^{1}H NMR: δ 6.39 (*s*, 2H, H-3, H-5), 4.18 (*s*, 4H, OCH_2CH_2O), 1.86 (*s*, 6H, Me). ^{13}C NMR: δ 186.4, 138.3 (2C), 135.5 (2C), 98.8, 65.3 (2C), 15.4 (2C). HRMS: calcd for $C_{10}H_{12}O_3$ [M]$^+$ 180.0786, found 180.0786.

(2E)-5-(2,6-*Dimethyl*-4,4-*ethylenedioxyl*-1-*hydroxycyclohexa*-2,5-*dienyl*)-3-*methylpent*-2-*en*-4-*yn*-1-*ol* **(9b)**. To a soln of *cis*-3-methylpent-2-en-4-yn-1-ol (2.88 g, 30 mmol) in THF (250 ml) under Ar at −78° was slowly added *n*-BuLi (1.6 M in hexane, 37.5 ml, 60 mml). After 30 min, ketone **8b** (3.35 g, 18.6 mmol) in 20 ml THF was added. The reaction was then allowed to warm to room temp. and stirred for 2 hr. After quenching with satd NH_4Cl (100 ml), the mixt. was extracted with ether (3 × 100 ml) and the combined organic layers were washed with satd NaCl, dried ($MgSO_4$) and concd. Flash chromatography (EtOAc–hexane 1:1) gave 4.16 g (81%) of **9b**. IR λ_{max} cm^{-1}: 3560 (OH), 1630, 1580 (C=C); ^{1}H NMR: δ 5.89 (*m*, 1H, H-2), 5.58 (*s*, 2H, H-3′, H-5′), 4.25 (*d*, 2H, J = 6.7 Hz, H-1), 4.03 (*s*, 4H, OCH_2CH_2O), 2.03 (*s*, 6H, C-7′, C-8′, Me), 1.85 (*d*, 3H, J = 1.3 Hz, C-3, Me). ^{13}C NMR: δ 140.5, 136.9, 123.0 (2C), 120.2 (2C), 100.4, 93.5, 82.5, 67.2, 65.1, 65.0, 61.1, 22.8, 18.0 (2C). HRMS: calcd for $C_{16}H_{20}O_4$ [M]$^+$ 276.1388, found 276.1382.

(2Z)-5-(2,6-*Dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2-*en*-4-*yn*-1-*ol* **(10)**. Ketal alcohol **9b** (303 mg, 1.1 mmol) was dissolved in acetone (10 ml) and 10% HCl (2 ml) was added. The soln was stirred at room temp. for 1 hr and concd to a syrup. The residue was partitioned between ether and 5% $NaHCO_3$, and the resulting aq. layer extracted with ether (2 × 10 ml). The ether layers were combined, dried ($MgSO_4$) and concd with further purification by recrystallization (ether–hexane) to give 221 mg (87%) of keto-alcohol **10**. Mp: 136–138°, IR λ_{max} cm^{-1}: 3600 (OH), 2240 (C≡C), 1715 (C=O), 1600, 1580 (C=C); ^{1}H NMR: (CD_3OD): δ 6.02 (*s*, 2H, H-3′, H-5′), 5.95 (*tq*, 1H, J = 6.6 Hz, H-2), 4.26 (*d*, 2H, J = 6.6 Hz, OCH_2), 2.20 (*s*, 6H, C-7′, C-8′, Me), 1.86 (*d*, 3H, J = 1.1 Hz, C-6, Me). ^{13}C NMR (CD_3OD): δ 187.5, 161.5, 139.4 (2C), 125.4 (2C), 119.6, 93.1, 83.9, 68.5, 61.4, 22.8, 19.0 (2C). HRMS: calcd for $C_{14}H_{16}O_3$ [M]$^+$ 232.1099, found 232.1093.

(2Z)-5-(2,6-*Dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2-*en*-4-*yn*-1-*al* **(11)**. Alcohol **10** (186 mg, 0.8 mmol) was dissolved in acetone (15 ml) and manganese dioxide (1 g) was added. The suspension was stirred at room temp. for 5 hr and filtered and the filtrate concd and purified by flash chromatography (EtOAc–hexane 1:5) to give aldehyde **11** (133 mg, 72%). IR λ_{max} cm^{-1}: 3550 (OH), 2230 (C≡C), 1730, 1715 (C=O), 1600, 1580 (C=C), ^{1}H NMR: δ 9.86 (*d*, 1H, J = 8.1 Hz, CHO), 6.19 (*dq*, 1H, J = 8.1 Hz, H-2), 6.01 (*s*, 2H, H-3′, H-5′), 2.21 (*s*, 6H, C-7′, C-8′, Me), 2.09 (*d*, 3H, J = 1.4 Hz, C-6, Me). ^{13}C NMR: δ 192.0, 185.4, 157.1, 140.8, 136.6 (2C), 125.6 (2C), 97.6, 81.4, 67.9, 24.5, 18.7 (2C). LRMS: 230 [M]$^+$, 212 [M − H_2O]$^+$.

Methyl-(2Z)-5-(2,6-*dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2-*en*-4-*yn*-4-*ynoate* **(12)**. To a soln of aldehyde **11** (1.012 g, 4.4 mmol) in MeOH (25 ml) were sequentially added MnO_2 (5 g), NaCN (0.441 g, 9 mmol) and acetic acid (0.25 ml). The reaction mixt. was stirred overnight at room temp. and filtered through a bed of Celite®, which was washed thoroughly with ether. The Et_2O solubles were combined with the filtrate, back extracted with 5% $NaCO_3$ and satd NaCl, dried ($MgSO_4$), and concd. Crude product was purified by flash chromatography (EtOAc–hexane 1:4) to afford keto-ester **12** (0.997 g, 84%). Recrystallization from ether–hexane gave crystals. Mp: 131–133°, IR λ_{max} cm^{-1}: 3590 (OH), 2240 (C≡C), 1715, 1700 (C=O), 1610, 1580 (C=C); ^{1}H NMR: δ 6.02 (*m*, 1H, H-2), 5.99 (*s*, 2H, H-3′, H-5′), 3.66 (*s*, 3H, OMe), 2.22 (*s*, 6H, C-7′, C-8′, Me), 1.98 (*d*, 3H, J

= 1.5 Hz, C-6, Me). ^{13}C NMR: δ 185.5, 165.1, 157.4, 133.5 (2C), 126.1, 125.5 (2C), 97.6, 84.0, 67.8, 51.5, 24.5, 18.7 (2C). HRMS: calcd for $C_{15}H_{16}O_4$ [M]$^+$ 260.1049, found 260.1075.

(2Z,4E)-5-(2',6'-*Dimethyl*-4',4'-*ethylenedioxy*-1'-*hydroxy*-*cyclohexa*-2',5'-*dienyl*)-3-*methylpent*-2,4-*dien*-1-*ol* (**13**). To a solution of ketal alcohol **9b** (2.34 g, 8.48 mmol) in THF (100 ml) at −78° was added dropwise a soln of Red-Al® (3.4 M in toluene, 5 ml, 17 mmol) in THF (50 ml). The solution was allowed to warm to room temp. and stirred overnight. The soln was poured on to ice-cold H_2O (500 ml) and the resulting mixt. extracted with ether (3 × 150 ml). The ether extracts were combined, dried ($MgSO_4$) and concd to afford crude **13** (in the 50% yield), this being directly used without further purification.

(2Z,4E)-5-(2,6-*Dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2,4-*dien*-1-*ol* (**14**). Crude ketal alcohol **13** (139 mg, 0.5 mmol) was hydrolysed with 2 ml of 10% HCl in acetone (10 ml) as described previously (see prepn of **10**). Flash chromatography (EtOAc–hexane 1:1) gave keto alcohol **14** (106 mg, 91%). IR λ_{max} cm^{-1}: 3600 (OH), 1720 (C=O), 1610, 1585 (C=C); ^{1}H NMR: δ 6.88 (*d*, 1H, *J* = 15.6 Hz, H-4), 6.00 (*s*, 2H, H-3', H-5'), 5.61 (*t*, 1H, *J* = 6.9 Hz, H-2), 5.31 (*d*, 1H, *J* = 15.6 Hz, H-5), 4,31 (*d*, 2H, *J* = 6.9 Hz, OCH_2), 1.95 (*s*, 6H, C-7', C-8', Me), 1.80 (*s*, 3H, C-6, Me). HRMS: calcd for $C_{14}H_{18}O_3$ [M]$^+$ 274.1256, found 276.1282.

(2Z,4E)-5-(2,6-*Dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2,4-*dien*-1-*al* (**16**). Aldehyde **15** (193 mg, 0.7 mmol) was hydrolysed with 10% HCl (1 ml) in acetone (5 ml) as previously described (see prepn of **10**). Flash chromatography (EtOAc–hexane 3:7) gave keto aldehyde **16** (136 mg, 84%). IR λ_{max} cm^{-1}: 3560 (OH), 1730, 1715 (C=O), 1660, 1660 (C=C); ^{1}H NMR: δ 9.97 (*d*, 1H, *J* = 8.3 Hz, CHO), 7.42 (*d*, 1H, *J* = 15.5 Hz, H-4), 6.02 (*s*, 2H, H-3', H-5'), 5.82 (*d*, 1H, *J* = 8.3 Hz, H-2), 5.72 (*d*, 1H, *J* = 15.5 Hz, H-5), 2.17 (*s*, 6H, C-7', C-8', Me), 1.99 (*d*, 3H, *J* = 1.0 Hz, C-6, Me). LRCIMS: 233 [M + 1]$^+$, 250 [M + 18]$^+$.

Methyl-(2Z,4E)-5-(2,6-*dimethyl*-4,4-*ethylenedioxy*-1-*hydroxycyclohexa*-2,5-*dienyl*)-3-*methylpent*-2,4-*dienoate* (**17**). Aldehyde **15** (504 mg, 1.8 mmol) was oxidized with MnO_2 (3 g), NaCN (196 mg, 4 mmol) and AcOH (0.1 ml) in MeOH (25 ml) as described previously (see prepn of **12**). Flash chromatography (EtOAc–hexane 1:4) afforded methyl ester **17** (0.997 g, 84%). ^{1}H NMR: δ 7.88 (*d*, 1H, *J* = 15.9 Hz, H-4), 5.65 (*s*, 1H, H-2), 5.65 (*d*, 1H, *J* = 15.9 Hz, H-5), 5.57 (*s*, 2H, H-3', H-5'), 4.01 (*s*, 4H, OCH_2CH_2O), 3.67 (*s*, 3H, OMe), 2.15 (*s*, 1H, OH), 1.91 (*d*, 3H, *J* = 1.1 Hz, C-6, Me), 1.75 (*s*, 6H, C-7', C-8', Me). ^{13}C NMR: δ 166.4, 149.8, 142.4 (2C), 138.7, 127.5, 123.1 (2C), 117.4, 100.8, 73.3, 65.1, 64.9, 51.0, 21.0, 17.3. LREIMS: 306 for $C_{17}H_{22}O_5$ [M]$^+$.

Methyl-(2Z,4E)-5-(2,6-*dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2,4-*dienoate* (**18**). Ketal methyl ester **17** (367 mg, 1.2 mmol) was hydrolysed with 10% HCl (2 ml) in acetone (10 ml) as described previously. Flash chromatography (EtOAc–hexane 1:1) gave keto methyl ester **18** as a solid, which was recrystallized from ether–hexane to give crystals (279 mg, 89%). Mp: 136.5–137.5°; IR λ_{max} cm^{-1}: 3590 (OH), 1715, 1700 (C=O), 1630, 1590 (C=C); ^{1}H NMR: δ 7.98 (*d*, 1H, *J* = 15.9 Hz, H-4), 6.00 (*s*, 2H, H-3', H-5'), 5.71 (*s*, 1H, H-2), 5.62 (*d*, 1H, *J* = 15.9 Hz, H-5), 3.66 (*s*, 3H, OMe), 1.96 (*s*, 6H, C-7', C-8', Me), 1.93 (*d*, 3H, *J* = 0.7 Hz, C-6, Me). ^{13}C NMR: δ 185.6, 166.4, 160.5, 149.2 (2C), 136.5, 128.7, 126.4 (2C), 118.4, 75.0, 51.2, 21.0, 18.1. HRMS: calcd for $C_{15}H_{18}O_4$ [M]$^+$ 262.1205, found 262.1205.

(2Z,4E)-5-(2,6-*Dimethyl*-1-*hydroxy*-4-*oxocyclohexa*-2,5-*dienyl*)-3-*methylpent*-2,4-*dienoic acid* (**19**). Methyl ester **17** (18 mg, 0.06 mmol) was dissolved in MeOH (2 ml) to which 8 ml of 0.5 N KOH was next added. The brown soln was stirred overnight at room temp. and concd *in vacuo* to remove the organic solvent. The residue was extracted with ether (5 ml) and then acidified with 10% HCl. The acidic soln was stirred at room temp. for 30 min and extracted with ether (3 × 10 ml). The ether layers were combined, dried (Na_2SO_4) and concd *in vacuo*. Recrystallization of the crude product from ether–hexane gave acid **6** (9 mg, 63%). ^{1}H NMR: δ 7.96 (*d*, 1H, *J* = 15.5 Hz, H-4), 6.03 (*s*, 2H, H-3', H-5'), 5.76 (*d*, 1H, *J* = 15.5 Hz, H-5), 5.74 (*s*, 1H, H-2), 1.99 (*s*, 6H, C-7', C-8', Me), 1.97 (*d*, 3H, *J* = 0.94 Hz, C-6, Me). HRMS: calcd for $C_{14}H_{14}O_3$ [M − H_2O]$^+$ 230.0925, found 230.0943.

Seed germination assay. The effect of compounds on seed germination was determined using cress seeds (*L. sativum* L.) imbibed at 25° in the dark. All experiments were replicated 3 ×, with 100 seeds per 9 cm Petri dish. The seeds were placed on 2 layers of Whatman no. 1 filter paper to which was added 3 ml of soln. The effect of the compounds on germination was determined over a range of 0.01–100 μM and the time for both radicle and shoot emergence was noted every 3 hr or as otherwise noted. Root emergence was defined as radicles equal or greater than seed length exhibiting positive geotropism. Shoot emergence was defined as being when the cotyledonary leaves had expanded and the testa was shed. The compounds were initially dissolved in acetone and made up to vol. with a final acetone concn of 0.1%.

Freeze tolerance assay. Bromegrass (*Bromus inermis* Leyss.) cell suspension cultures were grown in 50 ml of 0.5 Ericksson's (ER) media (containing B_5 micronutrient and vitamin solns) as described previously [19]. The divinyl analogues and ABA were initially dissolved in DMSO and made up to a final concn of 0.2% DMSO. The cells were incubated for seven days at 25° in 100 μM of each of the analogues. The affects of the analogues on freezing tolerance were compared to an untreated control and a culture treated similarly with 100 μM racemic ABA. Freezing resistance was measured as described in ref. [19] and the LT_{50} (50% killing temp.) values were determined by the TTC (2,3,5-triphenyl tetrazoliun chloride) reduction method [21]. Cell cultures were routinely screened for bacterial and fungal contamination by microscopy and plating on 2% YPD medium (2% glucose, 2.5% bactopetone, and 1% yeast extract). All experiments were replicated 3 ×.

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