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SYNTHESIS AND BIOLOGICAL ACTIVITY OF ALLENIC ANALOGUES OF ABSCISIC ACID

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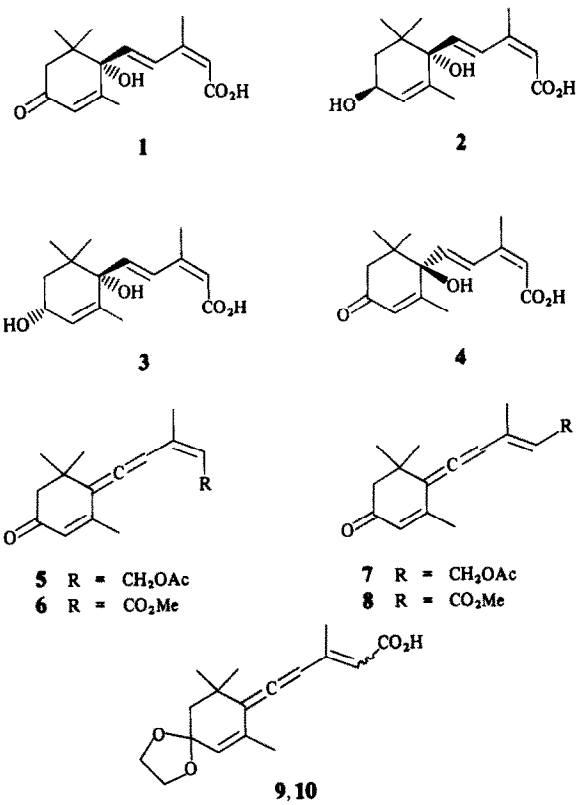
Key Word Index—*Lemna gibba*; Lemnaceae; abscisic acid; ABA; ABA analogue; allene; turion; growth inhibition.

Abstract—Novel allenic analogues of abscisic acid (ABA) were synthesized and tested for ABA activity. The allene functional group was introduced by one of two methods: base catalysed isomerization of an enyne to an enallene conjugated to a ketone, or reduction of a 2-butyn-1,4-diol to an allenic alcohol. A 3:1 mixture of (*E*)- and (*Z*)-3-methyl-5-(4′,4′-ethylenedioxy-2′,6′,6′-trimethylcyclohex-2′-en-1′-ylidene)-2,4,5-pentatrienoic acids strongly inhibited growth of axenic duckweed (*Lemna gibba*) causing a 50% reduction in frond number at 60 $\mu g l^{-1}$ over 7 days in continuous light. Racemic ABA caused the same inhibition at 130 $\mu g l^{-1}$. The mixture caused the production of turion-like structures, an effect known hitherto to be induced only by short photoperiods or by certain concentrations of ABA.

INTRODUCTION

We have synthesized novel allenic analogues of the plant hormone abscisic acid (ABA) (**1**) in which the sidechain is held rigidly in a pseudo-equatorial position. These molecules were designed to mimic a conformation that ABA may adopt when in an active site. Experimental results obtained in two assay systems had led one of us to propose [1] that the upper, or β, face of the ABA molecule, with the sidechain arranged in an equatorial relationship to the ring, interacts with a putative receptor. In one experiment with a saturable uptake carrier for ABA [2], the 1′,4′-*trans* diol **2** competes weakly with ABA, while the 1′,4′-*cis* diol **3** competes strongly. Our interpretation of these results was that the derivative with the hydroxyl group on the β face could not fit into the carrier site, while the compound with the hydroxyl group on the bottom face of the molecule could fit and elicit the response. As well, both *R*-ABA (**4**) and *S*-ABA (**1**) are active in bioassays for growth inhibition [3]. These two experimental results can be accommodated in a model in which ABA has the sidechain in a pseudo-equatorial orientation. This led us to synthesize analogues with two contiguous double bonds at the 4-5 and 5-1′ positions (using ABA numbering system [4]) affording compounds predicted to have the active conformation.

Application of abscisic acid and analogues [5] to various plant systems causes a variety of physiological responses of which growth inhibition is the most readily measured but one of the least specific. The long duration of experiments necessary to detect growth inhibition allows for activation and inactivation of candidate molecules and such experiments can suffer the complications of adventitious factors such as microbial metabolism and chemical instability [5]. Only the naturally occurring (+)-*S*-enantiomer of ABA causes rapid closure of stomata and this response can be detected within a few minutes of application.



We tested the activity of the analogues we had synthesized in stomatal closure assays and a growth assay using axenic duckweed (*Lemna gibba*) [6, 7]. Duckweed

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was used for these experiments because axenic cultures were available, the fronds have thin cuticles and thereby present a minimal barrier to the uptake, even of polar compounds. Their small size (2 × 4 mm), extremely simple structures and intimate contact with the solutions make the plants useful test systems for bioassays. In addition, the growth of duckweed is inhibited by ABA at very low concentrations and some strains produce specialized resting buds, 'turions', in response to ABA or short photoperiods. It is possible, therefore, to recognize compounds which have the same action as ABA and to compare their efficacy at low concentrations where solubility effects are minimal.

We now report the synthesis and biological activity of allenic analogues **5**–**10** all containing the allene group, but with variations of geometry of the double bond at C-2, oxidation level at C-1, and ethylenedioxy protecting group at C-4′.

RESULTS AND DISCUSSION

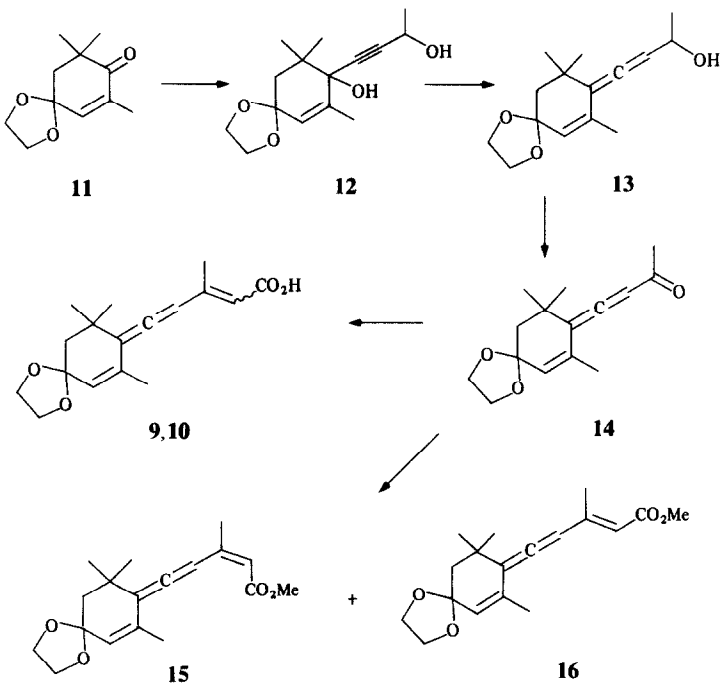
Synthesis

Two different strategies were used to synthesize the allenic analogues. Because of their lability, the ketal acids **9** and **10** were prepared by extending the sidechain of an allenic intermediate. The acetates **5** and **7** and the esters **6** and **8** were prepared by a more direct regiospecific route involving addition of the five carbon sidechain with subsequent introduction of the allene group.

Allenic abscisic acid **9**, **10** and ester analogues **6** and **8**, and ketal derivatives **15** and **16** were prepared in a sequence beginning with the monoethyleneketal of oxoisophorone (**11**) (Scheme 1). Alkylation of **11** with the dilithium salt of 3-butyn-2-ol in tetrahydrofuran afforded the butyndiol derivative **12** [8] in *ca* 50% yield. The crude product was converted into the allenic alcohol **13**, a diastereomeric mixture, by reduction with excess lithium aluminum hydride in ether according to Miki and Hara [9]. Oxidation to the methyl ketone **14** was effected with activated manganese dioxide in hexane in 55% isolated yield. Employing conditions reported by Nakanishi *et al.* [10] for Wadsworth Emmons modification of the Wittig reaction of a closely similar system, reaction of **14** with the ylide derived from *n*-butyllithium and trimethylphosphonoacetate in ether afforded a mixture of *cis* and *trans* unsaturated ketal esters **15** and **16**. Brief treatment of the individual isomers **15** and **16** with pyridinium *p*-toluenesulphonate in acetone–water [11] resulted in hydrolysis of the ketals affording the ketoesters **6** in 13% yield and **8** in 75% isolated yield. The synthesis of **6** and **8** was better effected by the second procedure described in detail in the Experiment section.

Reaction of the methyl ketone **14** with the dianion of diethylphosphonoacetic acid [12] afforded a 3:1 mixture of *trans* and *cis* ketal acids **9** and **10** in 40% yield. Attempted hydrolysis of the ketal mixture with pyridinium *p*-toluenesulphonate resulted in destruction of the *cis* isomer. Because of the instability of the *cis* acid the mixture of ketal acids was subjected to biological evaluation.

The *cis* allenic acetate **5** and allenic ester **6** were prepared from a common intermediate, the ketoalcohol **19** (Scheme 2). Alkylation of **17** with the dilithio salt of *Z*-3-methyl-pent-2-en-4-yn-1-ol afforded **18**. The compound was produced as a single stereoisomer [13, 14] with the sidechain axial, the methyl equatorial and the relative stereochemistry of the proton on C-2′ and the hydroxyl group on C-1′ on the same face of the ring. Hydrolysis of **18** gave quantitative conversion to the ketodiol **19**. Standard conditions successfully employed for dehydration of similar tertiary alcohols [15–17] gave



11 12 13 9,10 14 15 16

Scheme 1.

either no reaction or poor yields of the desired product. The required *syn* elimination of water from **19** was best effected by treatment with acetic anhydride, acetic acid and potassium hydrogen sulphate, affording the deconjugated ketone **20** in good yield. During the course of this reaction the primary alcohol was converted to the acetate. Isomerization to the desired allenic acetate **5** was effected smoothly by brief treatment of **20** with potassium carbonate in methanol.

For the preparation of the *cis* allenic ester **6**, the ketodiol **19** was oxidized in two steps [12] to the methyl ester **21**. The ester **21** was subjected to the dehydration, isomerization procedure used for the synthesis of the allenic acetate **5**, and the desired allenic ester was obtained in 42% yield from **19**.

The *trans* allenic acetate **7**, and allenic ester **8** were synthesized by an analogous route employing *E*-3-methylpent-2-en-4-yn-1-ol (Scheme 3). The known ketal alcohol **24** [12] was oxidized to **26** as in the *cis* series. In this case the dehydration reaction conditions afforded a mixture of the deconjugated acetylenic and conjugated allenic acetates **25** and **7**. The ester **8** was obtained as above from **26** in 50% yield.

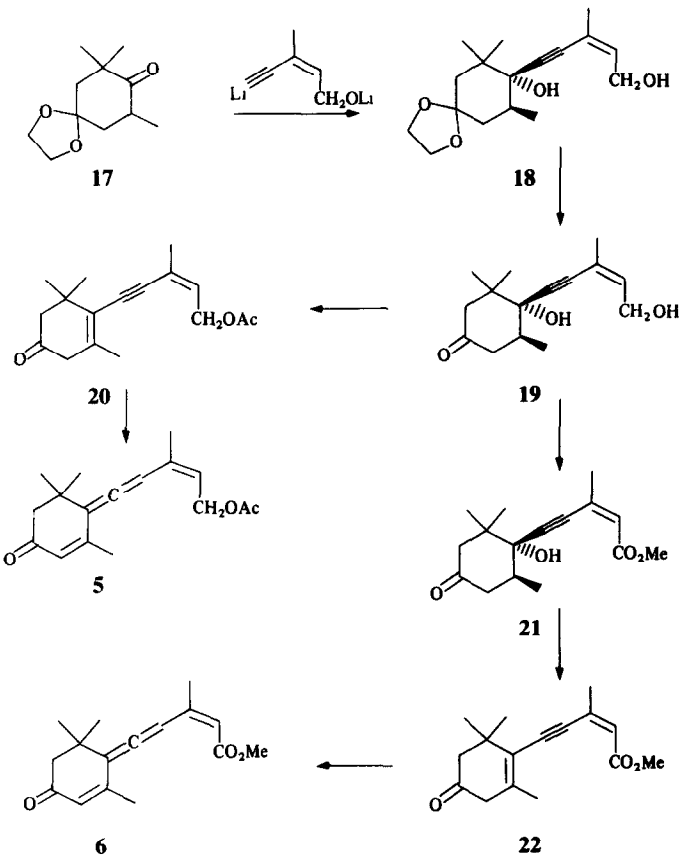
Table 1. The number of fronds of *L. gibba* growing axenically in a range of concentrations of each compound was compared with the number in control solutions (three replicates), the probit percentages of the control value were plotted against log concentration (during 7 days growth the relationship was linear and the 50% effective concentration was determined by interpolation)

Compounds tested	Concentration inhibiting Growth 50% ($\mu g l^{-1}$)
ABA	130
20	2500
5	3000
6	inactive*
7	inactive*
8	inactive*†
15	2200
16	2500
9 and 10	60

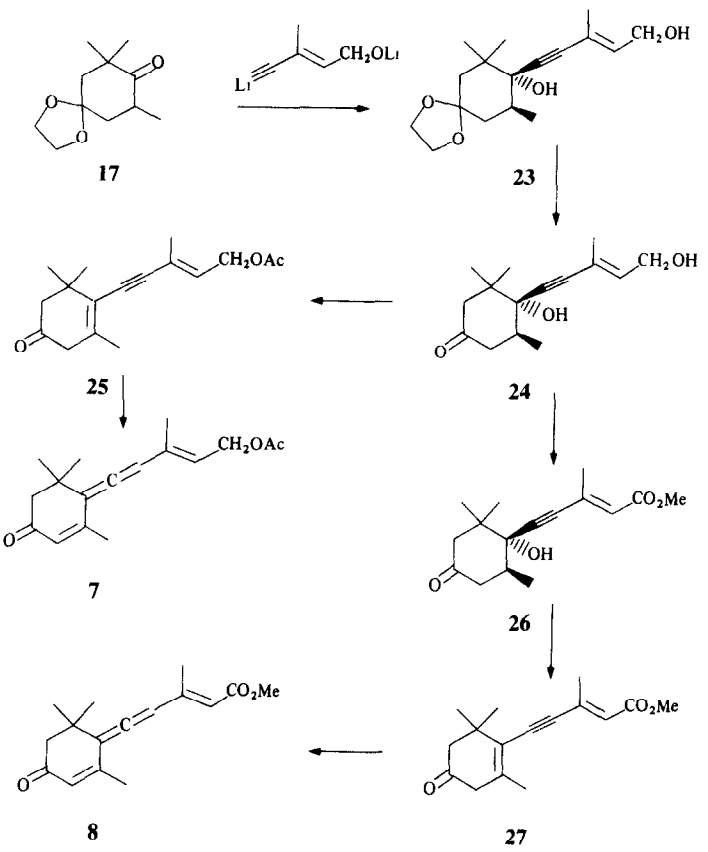
Control (all 10 fronds initially) mean = 234 fronds formed.
*Inactive indicates less than 50% growth inhibition at 10000 $\mu g l^{-1}$.
†Greater growth than control.

Effects on growth of Lemna gibba

The compounds were tested for their growth inhibitory activity on *L. gibba* growing under continuous light (Table 1). ABA and the mixture of **9** and **10** caused strong growth inhibition leading to senescence and death of the fronds. At intermediate concentrations (*ca* 10–30 $\mu g l^{-1}$) of the mixture **9** and **10**, and of ABA, the fronds developed novel structures which resembled the turions (dense, bulb-like, brown overwintering buds) previously ob-



Scheme 2.



Scheme 3.

served to be produced by duckweed in response to a narrow range of concentrations of ABA and to short photoperiods [6, 7].

The acids **9** and **10** were more active in the growth inhibition assay than either of the corresponding esters **15** and **16**. The *cis* and *trans* esters had comparable activity, possibly due to reversible photochemical isomerization occurring in the bioassay. The ketal esters **15** and **16** show growth inhibition, while the ketoesters **6** and **8** do not. The ketal protecting group may stabilize the molecule and enhance activity. As well, the allene **5** and acetylene **20** both reduced growth under the experimental conditions.

The activity of the acetylenic and allenic analogues of ABA in the growth inhibition test may be due the effects of the compounds themselves, or due to their conversion to active compounds, including ABA itself. With respect to the methyl ester **6** and **8**, we suspect that the esters were not hydrolysed in the plants, as we could not detect [^{14}C]ABA when [^{14}C]methyl ABA was fed to *Lemna*. In other experiments to be published elsewhere, we have subsequently established that ABA is formed from the allenic analogues **5** and **7** by *Lemna*. This can account, at least in part, for the biological effects observed. In the case of allenic acids **9** and **10** in which the ketone protected as the ketal, it is anticipated that the ketal group would be hydrolysed in the plant, possibly after the allene had been hydrated. We have observed that the ethylene ketal of ABA itself is remarkably labile, and is hydrolysed without the addition of acid catalyst. The pathway of conversion of the allenes to ABA is not known.

Effects on stomata

The analogues listed in Table 1 were supplied as aqueous solutions containing up to 1% ethanol to aid solubility (but had no detectable effect on controls). The solutions were fed to cut flowering stems of the sedge *Cyperus dispersa* held in a potometer so that the rate of water uptake could be monitored from the time the compounds entered the shoots. Compounds were tested at 100, 30, 10, 3, and 1 $\mu g l^{-1}$ and none showed a significant effect on water uptake, whereas ABA at all these concentrations produced a marked diminution of rate of water uptake within 5 min. Spray applications to the surface of the leaves and the placement of epidermal strips of *Commelina communis* on the surface of solutions of the analogues were also without effect on the stomata. It was concluded that the compounds themselves were inactive in this test.

CONCLUSION

All of the analogues studied were inactive in the short term tests. Either the allenes were inherently unable to meet the rigorous structural requirements of the stomatal receptors, or they required modification to become active and this did not occur within the time scale of the experiment (< 5 hr).

On the other hand, in longer term growth tests in *Lemna*, acids **9** and **10** inhibited the rate of frond multiplication strongly and induced the formation of turion-like structures which, hitherto, were produced uniquely by

ABA. Structures **9** and **10** may inhibit growth themselves. This is similar to the activity of (*R*)-ABA and other analogues of ABA which inhibit growth, but have no effect on stomata [4]. At this stage we cannot attribute the observed activity to the compounds themselves, their conversion into ABA or their metabolism to some other active derivatives. Further experiments to establish the mechanism of action of the allenic compounds are underway.

EXPERIMENTAL

Synthesis. Reactions which required dry conditions were performed in oven-dried (110° for more than 2 hr) glassware, under a positive pressure of Ar. Tetrahydrofuran (THF) was dried by distillation from Na and benzophenone.

^{1}H NMR spectra were recorded at 360 MHz, employing $CDCl_3$ as solvent with $CHCl_3$ as ref. For clarity, the conventional ABA numbering system is employed in assignments of peaks in the ^{1}H NMR spectra. Flash column chromatography was performed using E. Merck silica gel 60 (230–400 mesh). E. Merck precoated glass plates of silica gel 60 F254 (0.25 or 1.0 mm) were used in prep. TLC. E. Merck silica gel 60 F254 plates (0.2 mm) with aluminium sheet backing were used in analytical TLC. Ultraviolet active materials were detected under an UV lamp. The plates were then dipped into a soln of phosphomolybdic acid and slowly heated on a hot plate to visualize the spots. Prep. TLC was also performed on the Chromatotron (Harrison Research) with circular glass plates precoated with silica gel F254 (1, 2, or 4 mm), where the radial flow of eluent and sample were centrifugally accelerated. GC separations were carried out with DB-1701-30W capillary column (J and W Scientific, 30 m) and a FID. He at a flow rate of *ca* 2.5 cm min^{-1} was used as the carrier gas. The temp. of the column, the emergence times of peaks and their relative intensities are given in parentheses. GC-MS were obtained by using a DB-5 column (60 m), electron impact mode. Exact mass measurements were obtained with a VG ZAB-E-Q using peak matching mode by the Atlantic Research Laboratory of the National Research Council of Canada, Halifax Canada.

Butyn-1,4-diol (**12**). To a soln of 3-butyn-2-ol (3.1 ml, 40 mmol) in THF (75 ml) cooled in a dry ice–Me_2CO bath was added *n*-butyllithium (1.6 M in hexane, 53 ml, 85 mmol) at such a rate that the int. temp. was kept below −45°. After 0.5 hr, a soln of the monoketal of oxoisophorone (**11**) (7.8 g, 40 mmol) in THF (40 ml) was added dropwise to the thick white slurry. The reaction mixt. was allowed to warm to ambient temp. overnight. Water was added and the organic material extracted with Et_2O (× 3). The combined organic phases were washed with satd NaCl soln, dried over Na_2SO_4, filtered and the solvent evapd to afford 11.3 g crude product which was distilled bulb-to-bulb at 0.1 Torr, oven temp. 150–210°, 7.4 g. Trituration with Et_2O afforded crystalline ketal **12** (2.8 g) which gave a mp 130–140° (lit. mp 146°, [6]), a single peak on GC (200°, 10.9 min), and a single spot on TLC (R_f 0.3, Et_2O–hexane, 3:1); ^{1}H NMR δ: 5.33 (1H, *s*, H-3'), 4.52 (1H, *q*, J = 6.1 Hz, H-3), 3.90 (4H, *m*, $W_{1/2}$ 30 Hz, OCH_2), 1.8–1.95 (2H, *m*, H-5'), 1.88 (3H, *d*, J = 1.4 Hz, Me-2'), 1.42 (3H, *d*, J = 6.6 Hz, Me-3), 1.11 (3H, *s*, Me-6'), and 1.06 (3H, *s*, Me-6'); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1080; GC-MS *m/z* (rel. int.): 266 $[M]^+$ (1), 210 $[M-56]^+$ (57), and 43 (100); GC-MS of TMS derivative *m/z* 338 $[M]^+$ (1), 282 $[M-56]^+$ (71), 192 (73), 162 (68) and 87 (100).

The ketal proved extremely labile, and efforts to crystallize or purify the product by trituration resulted in extensive hydrolysis. In subsequent preparations, the crude alkylation product was carried through to the allenic alcohol **13**.

Allenic alcohol (**13**). To a soln of **12** (4.9 g, 18 mmol) in Et_2O (dry, 100 ml) at 0° was added $LiAlH_4$ (1.25 g) in portions. The mixt. was stirred at room temp. for 4 hr, and then the excess reagent was destroyed by careful addition of H_2O with cooling. The Et_2O phase was sepd and the aq. phase extracted with Et_2O (× 4). The combined ethereal phases were washed with satd NaCl soln, dried over K_2CO_3, filtered and the solvent evapd to afford 1.8 g of crude product. Chromatography over florisil, then over silica gel (Chromatotron, eluting with Et_2O–hexane, 3:1) afforded 2.2 g (8.8 mmol, 49%) of allene **13** which gave a single broadened peak on GC (200°, 6.7 min), a single spot on TLC (R_f 0.7, Et_2O–hexane, 3:1); ^{1}H NMR δ: 5.60 (1H, *d*, J = 5.5 Hz, H-4), 5.42 (1H, *br s*, H-3'), 4.33 (1H, *m*, H-3), 3.90 (4H, *m*, OCH_2), 1.80 (2H, *s*, H-5'), 1.73 (3H, *br s*, Me-2'), 1.27 (3H, *d*, J = 6.3 Hz, Me-3), 1.143, 1.138, 1.127, and 1.123 (6H, *s* of equal intensity C-6' CH_3); IR ν_{max}^{film} cm^{-1}: 3400 (br), 1960, 1640, 1200, and 1080; EIMS *m/z* (rel. int.); 250 (18), 235 (8), 206 (51), 205 (45), 191 (38), and 45 (100); UV λ_{max}^{hexane} nm: 251 (3400); Exact Mass Measurement: Calcd for $C_{15}H_{22}O_3$ 250.1563; Found 250.1576.

Allenic ketone (**14**). To a soln of the allenyl alcohol **13** (800 mg, 3.2 mmol) in hexane (100 ml) was added activated MnO_2 (5.5 g, 64 mmol). The mixt. was allowed to stir at room temp. overnight and then a further portion of activated MnO_2 (2.75 g, 32 mmol) was added and stirring continued for a further 2 hr. Filtration and evapn of the solvent afforded 540 mg of crude product. Chromatography over silica gel (Chromatotron, eluting with Et_2O–hexane, 1:1) yielded the pure allenyl ketone **14** (438 mg, 55%) which gave a single spot on TLC (R_f 0.5, Et_2O–hexane, 1:1) and a single peak on GC (200°, 7.2 min); ^{1}H NMR δ: 6.03 (1H, *br s*, H-3'), 5.54 (1H, *br s*, H-4), 3.95 (4H, *m*, OCH_2), 2.18 (3H, *s*, Me-3), 1.86 (2H, *s*, H-5'), 1.77 (1H, *d*, J = 0.7 Hz, Me-2'), 1.24 (3H, *s*, Me-6') and 1.19 (3H, *s*, Me-6'); IR ν_{max}^{film} cm^{-1}: 1920, 1665, 1630, 1200 and 1080; GC-MS *m/z* (rel. int.): 248 $[M]^+$ (7), 233 (3), 205 (15), and 44 (100); UV λ_{max}^{hexane} nm: 251 (3800) and Exact Mass Measurement: Calcd for $C_{15}H_{20}O_3$ 248.1407; Found 248.1413.

cis *and* trans *Ketal allenic esters* (**15** *and* **16**). To a soln of trimethylphosphonoacetate (0.77 ml, 4.75 mmol) in Et_2O (dry, 15 ml), at −78°, was added *n*-butyllithium (1.6 M soln in hexane, 2.4 ml, 3.8 mmol). The mixt. was allowed to warm to room temp. and after 1.0 hr, was cooled to −10° and a soln of the allenyl ketone **14** (466 mg, 1.9 mmol) in Et_2O (5 ml) was added. The mixt. was allowed to stir for 20 hr at room temp. before H_2O was added, and the mixt. extracted with Et_2O (× 3). The combined organic extracts were washed with satd NaCl soln, then dried over Na_2SO_4, filtered and the solvent evapd to afford 520 mg crude product. GC (250°) indicated that the mixt. contained four products: the *Z*-methyl ester **15** (5.1 min, 30%), the *E*-methyl ester **16** (5.3 min, 54%), the *Z*-butyl ester (7.9 min, 6%) and the *E*-butyl ester (8.5 min, 10%). The methyl esters were sepd from the butyl analogues by chromatography over silica gel (Chromatotron, eluting with hexane–Et_2O, 7:3) to afford a 2:1 mixt. of **7** and **6** (317 mg, 1.04 mmol, 55%) which gave a single spot on TLC (R_f 0.6, Et_2O–hexane, 1:1). The esters could be sepd by repeated elution on TLC (1 mm silica gel plate, eluting with Et_2O–hexane 4:1, × 5). The *cis* ketal ester **15** gave a single peak on GC (230°, 7.4 min) and ^{1}H NMR δ: 7.76 (1H, *s*, H-4), 5.65 (1H, *br s*, H-2) 5.46 (1H, *br s*, H-3'), 3.95 (4H, *m*, OCH_2), 3.69 (3H, *s*, OMe), 1.88 (3H, *s*, Me-3), 1.84 (2H, *s*, H-5'), 1.74 (3H, *s*, Me-2'), 1.18 (3H, *s*, Me-6'), and 1.16 (3H, *s*, Me-6'); IR ν_{max}^{film} cm^{-1}: 1920, 1710, 1620; GC-MS *m/z* (rel. int.): 305 (11), 304 (73), 289 (69), 273 (27), 257 (27), and 245 (100); UV λ_{max}^{hexane}: 277 mm (11 600).

The *trans* ketal ester **16** gave a single peak on GC (230°, 7.9 min) and ^{1}H NMR δ: 6.20 (1H, *br s*, H-4), 5.75 (1H, *br s*, H-2'), 5.49 (1H, *br s*, H-3'), 3.95 (4H, *m*, OCH_2), 3.69 (3H, *s*, OMe), 2.15 (3H, *br s*, Me-3), 1.84 (2H, *s*, H-5'), 1.73 (3H, *br s*, OCH_2-2'), 1.18 (3H, *s*, OMe), and 1.15 (3H, *s*, Me-6'); IR ν_{max}^{film} cm^{-1}: 1920, 1720,

1620; GC-MS *m/z* (rel. int.): 305 (13), 304 (86), 289 (48), 245 (43), 217 (46), 176 (63), 126 (62), 115 (84), 91 (94), and 77 (100); UV λ_{max}^{hexane} nm: 276 (11500).

cis *and* trans *Allenic acids* **9** *and* **10**. A soln of lithium diisopropylamide prepd from diisopropylamine (0.62 ml, 4.4 mmol) and *n*-butyllithium (1.6 M, 3.0 ml, 4.8 mmol) in THF (5 ml), was added dropwise to a soln of diethylphosphonoacetic acid [11] (462 mg, 2.2 mmol) in THF (5 ml) at −60°. After 0.5 hr, a soln of **14** (500 mg, 2.0 mmol) in THF (5 ml) was added dropwise and the temp. was maintained at −60° for 0.5 hr then allowed to rise to ambient temp. After stirring for 7 days the mixt. was poured into H_2O and extracted with Et_2O (×3). The combined extracts were washed once with satd NaCl soln and dried over Na_2SO_4, filtered and the solvent evapd to afford recovered **14** (285 mg). The aq. phase was acidified with NH_4Cl soln, and extracted with CH_2Cl_2 (×3). The combined extracts were dried over $MgSO_4$, filtered and the solvent evapd to afford a mixt. of ketal acids **9** and **10** (190 mg) which was passed through a short silica plug eluting with Et_2O to afford 102 mg of a mixt. of **9** and **10** (41% yield based upon recovered starting material). GC (230°) of the mixt. treated with CH_2N_2 gave two peaks corresponding to the ketal methyl esters **16** and **15** with a ratio of *trans* to *cis* of 3:1. Integration of the vinyl protons in the ^{1}H NMR indicated that the *trans* predominated over the *cis* by a ratio of 2.5:1.0: δ: 7.75 (*s*, 0.4H), 6.23 (*s*, 1.0H), 5.77 (*s*, 1.0H), 5.68 (0.4H), 5.50 (*s*, 1.0H) and 5.46 (*s*, 0.4H).

cis *enynol* **18**. To a soln of *Z*-3-methylpent-2-en-4-yn-1-ol (2.9 g, 30 mmol) in THF (dry, 90 ml) at −55° was added *n*-butyllithium (39 ml, 63 mmol). After 15 min a soln of ketone **17** (4.95 g, 24 mmol) in THF (dry, 70 ml) was added. The soln was allowed to warm to room temp. and after 1.0 hr the mixt. was poured into H_2O and extracted with Et_2O (×3). The combined organic phases were washed with satd NaCl soln and dried over Na_2SO_4. The solvent was evapd at red. pres. and the product distilled bulb-to-bulb under vacuum (0.1 Torr) with an oven temp. of 180–200° affording 5.0 g (71% yield) of **18** that gave a single spot on TLC (R_f 0.2, Et_2O–hexane, 1:1); ^{1}H NMR δ: 5.80 (1H, *tq* J = 6.7, 1.5 Hz, H-2), 4.22 (2H, *dd*, J = 6.7, 1.0 Hz, H-1), 3.8 (4H, *m*, $[OCH_2]_2$), 2.15 (1H, *m*, H-2'), 1.82 (3H, *d*, J = 1.2 Hz, Me-3), 1.76 (1H, *d*, J = 14.3 Hz, H-5'$_{eq}$) 1.62 (2H, *m*, H-3'), 1.48 (1H, *d*, J = 14.3 Hz, H-5'$_{ax}$), 1.07 (3H, *s*, Me-6'), 1.05 (3H, *s*, Me-6') and 1.00 (3H, *d*, J = 6.6 Hz, Me-2'); IR ν_{max}^{film} cm^{-1}: 3400, 2240; GC-MS of mono TMS ether *m/z* (rel. int.): 366 $[M]^+$ (2), 348 (2), 129 (100).

Ketone **19**. To a soln of ketal **18** (2.6 g, 8.8 mmol) in THF (20 ml) was added dil HCl (20 ml). After 2 hr satd $NaHCO_3$ soln was added and the product was extracted with Et_2O (×3). The combined organic phases were washed with satd NaCl soln, dried over Na_2SO_4, and evapd to afford the ketone (2.2 g, 100% yield) which gave a single spot on TLC (R_f 0.3, Et_2O–hexane, 3:1); ^{1}H NMR δ: 5.92 (1H, *tq*, J = 6.5, 1.5 Hz, H-2), 4.32 (2H, *d*, J = 6.5 Hz, H-1), 2.64 (1H, *d*, J = 14.3 Hz, H-5'$_{ax}$), 2.3 (3H, *m*, H-2', H-3') 2.1 (1H, *br d*, J = 14.3 H-5'$_{eq}$), 1.91 (3H, *q*, J = 1.5 Hz, Me-3), 1.20 (3H, *s*, Me-6'), 1.14 (3H, *m*, Me-2'), and 0.98 (3H, *s*, Me-6'); IR ν_{max}^{film} cm^{-1}: 3400, 1690; GC-MS *m/z* 250 $[M]^+$ (1), 232 (3), 179 (22), 165 (29) and 106 (100); UV λ_{max}^{MeOH} nm: 240 (3000).

cis *Enyne acetate* (**20**). To a soln of ketodiol **19** (2.0 g, 8.0 mmol), HOAc (24 ml) and Ac_2O (16 ml) was added $KHSO_4$ (1.1 g, 8.0 mmol). The mixt. was stirred and heated at 70° under an Ar atmosphere for 1.75 hr, then cooled to room temp. Water was added and the mixt. was extracted with Et_2O (×3). The combined organic phases were washed with satd NaCl soln, then carefully with $NaHCO_3$ soln, adding solid $NaHCO_3$ until foaming subsided. The Et_2O layer was washed with satd NaCl soln, then dried over Na_2SO_4, and the filtrate evapd at red. pres. to afford the cyclohexenone **20** (1.76 g, 76%). The crude product gave a single spot on TLC (R_f 0.4, Et_2O–hexane, 1:1); IR ν_{max}^{film} cm^{-1}: 2160, 1730, 1710 and 750; UV λ_{max}^{MeOH} nm: 272 (10000); ^{1}H NMR δ: 5.77 (1H, *br t*, J = 6.9 Hz, H-2), 4.77 (2H, *d*, J = 6.9 Hz, H-1), 2.91 (2H, *s*, H-3'), 2.40 (2H, *s*, H-5'), 2.03 (3H, *s*, OCMe), 1.95 (3H, *s*, Me-3 or Me-2'), 1.94 (3H, *s*, Me-3 or Me-2') and 1.16 (6H, *s*, Me-6'); GC-MS *m/z* (rel. int.): 274 $[M]^+$ (22), 232 (9), 231 (10), 214 (100), and 172 (43).

cis *Enynoic ester* (**21**). To a soln of ketodiol **19** (2.0 g, 8.0 mmol) in Me_2CO (80 ml) was added MnO_2 (13.9 g, 160 mmol). The mixt. was stirred at room temp. for 1.0 hr before the MnO_2 was removed by filtration. The solid residue was rinsed with Me_2CO which was combined with the filtrate. Evapn of the solvent gave the crude aldehyde (1.68 g) which was oxidized to the ester without purification. The product could be crystallized from Et_2O, mp 126–127°. The crude aldehyde (1.68 g, 6.8 mmol) obtained above was dissolved in MeOH (7.5 ml). MnO_2 (8.9 g, 109 mmol), NaCN (800 mg, 16.3 mmol) and HOAc (390 μl, 6.8 mmol) were added. The mixt. was stirred for 2 hr before it was filtered through Celite. The residue was rinsed with MeOH and combined with the filtrate, and solvent evapd. The residue was partitioned between Et_2O and H_2O. The H_2O phase was extracted twice with Et_2O. The combined organic phases were washed twice with H_2O, satd NaCl soln and then dried over Na_2SO_4 and evapn of the solvent afforded 1.43 g crude product. Purification by chromatography over silica gel gave ester **21** 930 mg (42% from alcohol **19**) as a single spot on TLC (R_f 0.6, Et_2O–hexane, 3:1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1710, 1620; ^{1}H NMR δ: 6.02 (1H, *q*, J = 1.5 Hz, H-2), 3.68 (3H, *s*, OMe), 2.85 (1H, *d*, J = 14.3 Hz, H-5'$_{ax}$), 2.49 (1H, *d*, J = 14.3 Hz, H-5'$_{eq}$), 2.1–2.4 (3H, *m*, H-2', H-3'), 2.06 (3H, *d*, J = 1.5 Hz, Me-3), 1.57 (3H, *s*, Me-6'), 1.16 (3H, *d*, J = 6.2 Hz, Me-2') and 0.99 (3H, *s*, Me-6'); GC-MS *m/z* (rel. int.): 278 $[M]^+$ (4), 219 (46) and 137 (100).

cis *Allenic acetate* (**5**). To a soln of **20** (100 mg, 0.4 mmol) in MeOH (10 ml) at 0° was added 10 mg solid K_2CO_3. After 20 min H_2O was added to the dark soln, and the organic product extracted with Et_2O (×3). The combined Et_2O phases were washed once with NaCl soln, dried over Na_2SO_4, filtered, and the filtrate evapd. Chromatography of the crude product (Chromatotron, eluting with Et_2O–hexane 1:1), afforded the *cis* allenic acetate **5** 48 mg, 48% yield. ^{1}H NMR δ: 6.67 (1H, *s*, H-4), 5.89 (1H, *m*, H-3'), 5.50 (1H, *t*, further split, J = 7.3 Hz, H-2), 4.70 (2H, *d*, J = 7.3 Hz, H-1), 2.38 (2H, AB quartet, J = 16 Hz, H-5'), 2.06 (3H, *s*, OCMe), 1.96 (3H, *m*, Me-3'), 1.80 (3H, *m*, Me-3), 1.19 (3H, *s*, Me-6'), 1.18 (3H, *s*, Me-6'); IR ν_{max}^{film} cm^{-1}: 1920, 1730, 1655, and 1590; UV λ_{max}^{MeOH} nm: 284 (16900); MS (solid probe) *m/z* (rel. int.): 274 $[M]^+$ (4), 232 (19) and 214 (94).

cis *Enynoic ester* (**22**). Using the procedure employed to dehydrate compound **19**, treatment of **21** (2.0 g, 7.2 mmol) with HOAc (21.6 ml), Ac_2O (14.4 ml) and $KHSO_4$ afforded, after work-up and chromatography, 680 mg of **22** (38% yield) which gave a single spot on TLC (R_f 0.85, Et_2O–hexane, 3:1); ^{1}H NMR δ: 5.94 (1H, *q*, J = 1.4 Hz, H-2), 3.71 (3H, *s*, OMe), 2.93 (2H, *s*, H-3'), 2.4 (2H, *s*, H-5'), 2.09 (3H, *d*, J = 1.4 Hz, Me-3), 2.06 (3H, *s*, Me-2') and 1.21 (6H, *s*, *gem* Me); GC-EIMS *m/z* (rel. int.): 260 $[M]^+$ (16) and 245 (100).

cis *Allenic ester* (**6**). Isomerization of **22** (680 mg, 2.6 mmol) with MeOH (20 ml) and K_2CO_3 afforded 600 mg **6** which gave a single spot in TLC (R_f 0.8, Et_2O–hexane, 3:1); ^{1}H NMR δ: 7.97 (1H, *s*, H-4), 5.90 (1H, *br s*, H-2 or H-3'), 5.73 (1H, *br s*, H-2 or H-3'), 3.72 (3H, *s*, OMe), 2.40 (2H, AB *quartet*, J = 15.9 Hz, H-5'), 1.96 (3H, *s*, Me-2' or Me-3), 1.92 (3H, *s*, Me-2' or Me-3), 1.20 (3H, *s*, *gem* Me), 1.19 (3H, *s*, *gem* Me); IR ν_{max}^{film} cm^{-1}: 1920, 1700, 1660, 1620 and 1590; GC-CIMS *m/z* (rel. int.): 261 $[M+1]^+$ (7), 260 $[M]^+$ (49), 245 (100) and 185 (46); GC-EIMS *m/z* (rel. int.): 260 $[M]^+$ (55) and 245 (100); UV λ_{max}^{hexane} nm: 287 (14500); Exact Mass Measurement: Calcd for $C_{16}H_{20}O_3$ 260.1407; Found 260.1414.

trans *Enyne acetate* (**25**) *and* trans *allenic acetate* (**7**). A soln of **24** (570 mg, 2.3 mmol) and $KHSO_4$ (*ca* 20 mg) in HOAc (3.0 ml) and Ac_2O (2.0 ml) was heated under Ar for 2.5 hr at 100°. The soln was cooled to room temp., H_2O was added, and the product extracted with Et_2O (×3). The combined organic phases were washed first with satd $NaHCO_3$ soln, then with NaCl soln and dried over Na_2SO_4. Evapn of the solvent afforded an oil (483 mg) which was chromatographed over silica gel eluting with Et_2O–hexane (3:7), yielding two products pure: **7** (48 mg, 9%) and **25** (148 mg, 23%). The less polar product **25** gave a single spot on TLC (R_f 0.4, Et_2O–hexane, 1:1); ^1H NMR δ: 5.90 (1H, *tq*, $J=7.2$, 1.4 Hz, H-2), 4.66 (2H, *d*, $J=7.2$ Hz, H-1), 2.89 (2H, *s*, H-3'), 2.39 (2H, *s*, H-5'), 2.05 (3H, *s*, OCMe), 1.90–1.93 (6H, Me-3, Me-2'), 1.15 (6H, *s*, Me-6'); GC-MS *m/z* (rel. int.): 274 $[M]^+$ (22), 214 (100).

The allenic product **7** gave a single spot on TLC (R_f 0.3, Et_2O–hexane, 1:1); ^1H NMR δ: 6.31 (1H, *br s*, H-2'), 5.89 (1H, *t*, $J=1.2$ Hz, H-2), 5.61 (1H, *br t*, $J=7.0$ Hz, H-4'), 4.68 (2H, *d*, $J=7.0$ Hz, H-5'), 2.38 (2H, *br s*, H-6), 1.94 (3H, *d*, $J=1.2$ Hz, Me-3), 1.74 (3H, *br s*, Me-2'), 1.17 (3H, *s*, Me-5), and 1.15 (3H, *s*, Me-5); IR ν_{max}^{film} cm^{-1}: 1910, 1730, 1650, and 1590; GC-MS *m/z* (rel. int.): 274 (.5), 232 (7), 214 (100) and 199 (49); UV λ_{max}^{hexane} nm: 267 (24 400).

trans *Ester* (**26**). Using the conditions and reagents employed in the *cis* series, *trans* alcohol **24** was oxidized first to the aldehyde and then to the ester **26**, which gave a single spot on TLC (R_f 0.7, Et_2O–hexane, 3:1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1705, 1610; ^1H NMR δ: 6.09 (1H, *q*, $J=1.5$ Hz, H-2), 3.71 (3H, *s*, OMe), 2.62 (1H, *d*, $J=1.5$ Hz, H-5'$_{ax}$), 2.28–2.32 (3H, *m*, H-3', H-2'), 2.10 (1H, *dd*, $J=14.3$, 2.0 Hz, H-5'$_{eq}$), 1.19 (3H, *s*, Me-6'), 1.13 (3H, *m*, Me-2') and 0.98 (3H, *s*, Me-6'); GC-MS *m/z* (rel. int.): 278 (9), 247 (7), 219 (18) and 137 (100); UV λ_{max}^{MeOH} nm: 254 (20 500).

trans *Ester* (**27**) *and* trans *allenic ester* (**8**). Dehydration of *trans* ester **26** as described for **20** gave **27** (67%) that gave a single peak in GC (210–240° at 4° min^{-1}, 5.4 min) and a single spot on TLC (R_f 0.8, Et_2O–hexane, 3:1); ^1H NMR δ: 6.05 (1H, *br s*, H-2), 3.71 (3H, *s*, OMe), 2.92 (2H, *s*, H-3'), 2.40 (3H, *s*, Me-2'), 1.95 (2H, *s*, H-5'), 2.34 (3H, *d*, $J=1.2$ Hz, Me-3), 1.16 (6H, *s*, Me-6'). Isomerization of the enyne ester **27** and purification by chromatography (Chromatotron, cluting with Et_2O–hexane 2:3) gave the allenic ester **8** in 85% yield, as a single peak on GC (230°, 6.4 min); ^1H NMR δ: 6.34 (1H, *br s*, H-4), 5.92 (1H, *s*, H-3'), 5.81 (1H, *s*, H-2), 3.71 (3H, *s*, OMe), 2.40 (2H, *s*, H-5'), 2.19 (3H, *s*, Me-3), 1.95 (3H, *s*, Me-2'), 1.20 (3H, *s*, Me-6'), and 1.17 (3H, *s*, Me-6'); IR ν_{max}^{film} cm^{-1}: 1910, 1700, 1650, 1605 and 1590; GC-MS *m/z* (rel. int.): 261 (9), 260 $[M]^+$ (60), 245 (100), 185 (47) and 161 (76); UV λ_{max}^{hexane} 285 (12 500); Exact Mass Measurement: Calcd for $C_{16}H_{20}O_3$ 260.1407; Found 260.1411.

Culture of plant material. Axenic duckweed (*Lemna gibba*) was grown under continuous light at 25° on the medium described in ref. [16]. A concentrate of major nutrients was prepd which gave the following mixt. when diluted ×100 to 1.0 l: KH_2PO_4, 503; KNO_3, 889; $Ca(NO_3)_2$, 638; $MgSO_4$, 244 $mg\,l^{-1}$, 4.1 g sucrose and 3.0 g tartaric acid were dissolved in 850 ml H_2O together with 10 ml of the stock soln. Then, 1 ml each of stock solns containing: 9.0 $mg\,l^{-1}$ DTA.4NA salt and 3.3 $mg\,l^{-1}$ $FeCl_3$ were added together with 1 ml of a microelement stock soln (prepd freshly every few weeks) [17] which gave the following final concns in $mg\,l^{-1}$: H_3BO_4, 2.9; $ZnSO_4$, 0.15; Na_2MoO_4, 0:11; $CuSO_4$, 0.06; $MnCl_2$ 3.0. The pH was adjusted to 4.6 with KOH or HCl, the vol. made up to 1 l and the soln was autoclaved at 150 KPa, 20 min, 110°. Cultures were started by placing 5–10 fronds in 100 ml of medium in a sterile 250 ml conical flask stoppered with cotton wool and grown for 8–12 days before subculturing.

Growth bioassays. The growth inhibitory activity of the compounds was assayed by placing the required number of fronds, usually 4 or 10, in a sterile 25 ml glass flask or tube containing 10 ml medium. A measured quantity of the test compound was sterilized by dissolving it in EtOH–H_2O (7:3). The solns were allowed to stand for 10 min and then were prepd in the dilution series. Equal vols of the solns were injected into the growth flasks which were stoppered with cotton wool. The final EtOH concns never exceeded 1% and no detectable changes were observed in flasks which contained 1% EtOH only with untreated blanks. The concn at which each compound produced a 50% reduction in frond number at 7 days was obtained by interpolation.

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