Phytochemistry, Vol. 32, No. 4, pp. 827–832, 1993
Printed in Great Britain.

0031-9422/93 $6.00+0.00

METABOLISM OF ALLENIC ANALOGUES OF ABSCISIC ACID

B. V. MILBORROW* and SUZANNE R. ABRAMS†

School of Biochemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia; †Plant Biotechnology Institute, National Research Council Canada, 110 Gymnasium Road, Saskatoon, Saskatchewan, Canada S7N 0W9

(*Received* 2 *June* 1992)

Key Word Index—*Lemna gibba*; *Riccia fluitans*; abscisic acid; allene; ABA analogue; metabolism.

Abstract—We have already reported that some allenic analogues of abscisic acid inhibited growth of duckweed (*Lemna gibba* and *L. paucicostata*) in axenic culture, and other plants. The duckweed produced turion-like structures in the presence of these analogues when growing in continuous light. The induction of turions has hitherto been caused only by ABA or short photoperiods. Deuteriated, allenic analogues were supplied to axenic cultures of the *L. gibba*, for seven days and the ABA in the media and in the fronds was found to contain deuterium, thereby establishing that allenic analogues had been converted into ABA. The oxygen atom at C-1′, the addition of which is required during the formation of ABA from the allenic analogues, is derived from the medium. This was determined by mass spectrometry of the ABA formed in experiments in which the *L. gibba* fronds had been supplied with [$^2H_2$]AB allenic alcohol acetate and grown in [^{18}O]H_2O (30 atoms %). The ABA was found to contain ^{18}O at C-1′. The 2-*trans* allenic AB alcohol acetate was converted into 2-*trans*-ABA and 2-*trans*-AB aldehyde. Thus the growth inhibition and morphological effects observed when the plants were grown in the presence of the allenic analogues can be attributed to the ABA formed from them.

INTRODUCTION

We had previously synthesized a number of allenic analogues of abscisic acid (ABA) (**1**) [1] to test the hypothesis that a similar molecule to ABA, which had its sidechain held rigidly in what was predicted to be active conformation, would have the same effects on plants as ABA. The allenes were found to be potent inhibitors of the growth of axenic duckweed (*Lemna gibba*) and to cause the fronds to form turion-like structures, that were very similar to those produced by a narrow range of concentrations of ABA [2, 3]. This occurred in spite of the plants' being grown under continuous light. On the other hand, the allenic derivatives were inactive in short term bioassays (< 1 hr) on stomata of the sedge *Cyperus diffusus* when supplied as aqueous solutions to cut stems.

We deemed it necessary to establish if the allenic compounds had been converted into ABA or whether the observed responses could be attributed to the allenes themselves. This was accomplished by preparing allenic compounds (**2**, **3**) labelled into a ^2H atom at C-3′ and C-4 and, latterly, $^2H_3$ at C-7′. We now describe our studies on the biotransformation of these compounds into ABA.

RESULTS AND DISCUSSION

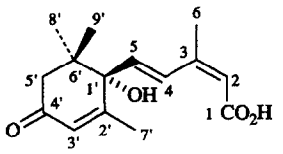
The 2-*Z*,*cis* double bond of ABA isomerizes readily in light in solution and in the presence of plant material the process is even more rapid. The synthetic methods employed to make the allenic analogues of ABA give both 2-*cis*- and 2-*trans*-isomers, so both were bioassayed. It was expected that they would be interconverted by the illumination of the solutions in which the *Lemna* fronds were grown, but the 2-*trans* isomers were much less active and a subsequent test showed that when the 2-*trans* allenic alcohols (**2**, **3**) were illuminated in daylight for three days no detectable amounts (< 2%) of the 2-*cis* isomers were found. Consequently, the weak inhibition of growth caused by the 2-*trans* allenic analogues was attributed to the isomerization of 2-*trans*-ABA formed from them to ABA, not by direct isomerization of any allenic compound.

The 4′-ketals of some of the allenic analogues were prepared as the presence of an unprotected 4′-keto group caused the compounds to break down. We had previously found that ABA-4′-ketal spontaneously hydrolysed overnight so it was reasonable to include ketals in the studies of transformations.

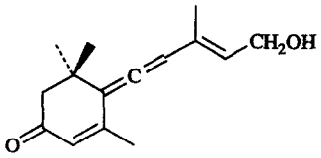
The instability, particularly of 2-*cis*-allenic acid analogues, led us to synthesize allenic alcohols and alcohol acetates as compounds with these C-1 groups generally penetrate cells readily and AB alcohol and AB aldehyde are rapidly oxidized to ABA by plants. We made 2-*cis*- and 2-*trans*-allenic analogues to see if they were each metabolized to ABA.

Although the allenic ketals (**4**, **5**) were the most potent inhibitors of growth, the allenic 2-*cis* alcohol (**6**) and allenic alcohol acetate (**2**) also displayed growth inhibitory activity, so metabolism was probably involved. It was

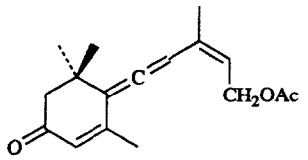
*Author to whom correspondence should be addressed.



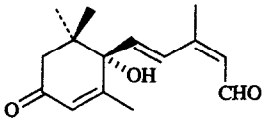
1 (+) -*S*-Abscisic acid (Revised numbering)



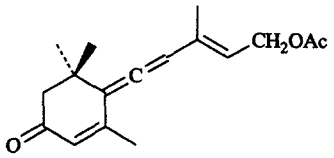
6



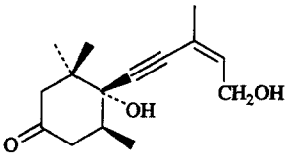
2



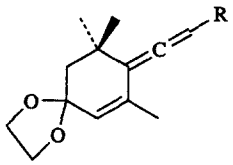
7 (+) -*S*-Abscisic aldehyde

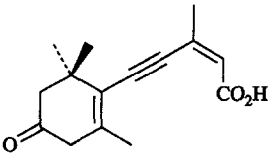


3

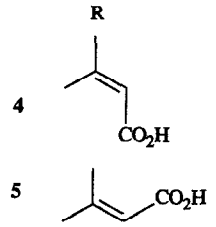


8





9



4

5

necessary to establish whether the allenic analogues were active *per se* or were converted into ABA.

Allenic alcohols were made with a deuterium atom at C-3′ and at C-4. These are the positions in which deuterium can be incorporated during the synthesis by carrying out the base-mediated rearrangement of the acetylenic intermediate to the allene with deuteriated methanol as the solvent of the reaction [1]. Compounds so labelled have the advantage that, if metabolized to ABA and then methylated chemically, the sidechain of Me ABA formed from them is readily identified by negative ion CI-GC mass spectrometry [4–6] as a major ion at *m/z* 141.

The ring of ABA, with the 1′-hydroxyl group, gives a fragment ion at *m/z* 152 [5]. The dideuterio samples would give fragment ions of 142 and 153. Thus these two ions give important information about the location of heavy isotopes in the molecule. In the experiments in which the *Lemna* plants were grown on $H_2{}^{18}O$ and were supplied with allenes, only very small quantities of medium and less than 1 g of fronds could be used. The extremely small quantities of ABA formed under those conditions required that the method of greatest sensitivity be employed to detect the presence of any label incorporated, namely the formation of the pentafluorobenzyl ester by NICI-GC mass spectrometry [5]. Although attomolar quantities can be detected, almost all the ion current is carried by the ABA-H ion so less precise information can be obtained about the position of the heavy atoms.

The 7′-$^2H_3$ labelled versions of **2** and **3** were also made. The ABA formed from these was methylated and analysed by PIEIGC mass spectrometry where the major fragment ion *m/z* 190 carries most of the ion current and the *m*+3/*z* peak at 193 can be clearly seen after the compounds have been converted into ABA (Fig. 1A) by *Lemna*.

The results in Table 1 established that labelled ABA was formed from **2** and labelled 2-*trans*-ABA from **3** by fronds of *L. gibba*. This required two oxidations at C-1 and reaction at C-5 and C-1′. $^2H_2$ labelled abscisic aldehyde (**7**) and 2-*trans*-abscisic aldehyde were also detected in the extract of the medium when **2** and **3**, respectively, were supplied. The amounts were similar to the amounts of ABA present.

The conversion of the allenic structure into the *cis,trans*-3-methyl penta-2,4-dienoic acid side chain and OH-1′ of ABA is formally a hydration. Consequently, $^2H_2$ allene was supplied to four axenic *L. paucicostata* fronds in $H_2{}^{18}O$ in darkness in the expectation that hydration of the C-5 double bond would result in ABA with an ^{18}O atom at C-1′. The ABA formed was labelled with one or two atoms of ^{18}O. The compounds were held in an aqueous extract during the isolation procedure for more than 24 hr so any ^{18}O taken up in $H_2{}^{18}O$ by

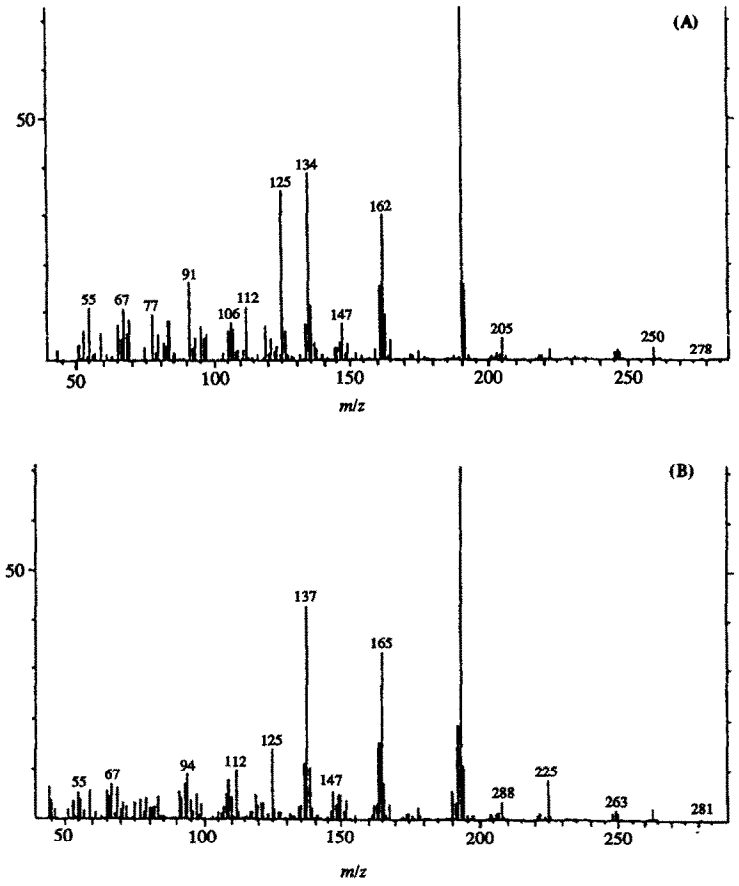


Fig. 1. Mass spectra (EIGC-MS): (A) Me ABA, (B) Me ABA formed from [C-7-$^2H_3$] compound **2** by *Lemna* fronds. The small peak at *m/z* 190 is the endogenous, unlabelled material which represents 5% of the total Me ABA. The relative stereochemistry of **8**, **10** and **11** is shown but the compounds used were racemic.

Table 1. Formation of ABA and 2-*trans*-ABA from allenic and acetylenic precursors

Deuterium labelled compound supplied	**2**	**3**	**8**	**9**
Yeast*	No ABA	No ABA	No ABA	No ABA
*Cercospora rosicola**	No labelled ABA	No labelled ABA	Not tested	Not tested
*Lemna paucicostata**	[^{2}H]ABA (+):(−)=1:1 in ABA *cis*:*trans*=10:7	[^{2}H]-2-*trans*-ABA	No labelled ABA	Trace [^{2}H]ABA
Riccia fluitans	[^{2}H]ABA	[^{2}H]-2-*trans*-ABA	No labelled ABA	Trace [^{2}H]ABA

The presence of 7′-deuterium labelled ABA was detected by EIPIGC mass spectrometry of the ABA in yeast and *R. fluitans* and 3′,4 deuterium labelled ABA as pentafluorobenzyl ABA in *C. rosicola* and *L. paucicostata* by NICIGC mass spectrometry.

*Axenic cultures were used.

exchange at C-4′ would have been lost from the molecule of ABA: its 4′-oxygen atom is rapidly lost by exchange with the medium [7]. The presence of the two deuterium atoms, at C-3′ and C-4 in the allene supplied, permits discrimination between any ABA formed *de novo* and containing an ^{18}O atom and the ABA derived from the dideuterio allene because the latter would show a parent ion four mass units greater than normal material.

It has been reported previously [8] that liverworts do not contain ABA. However, the *Riccia fluitans* used in this investigation was not axenic and had been grown in an aquarium with higher plants so could have absorbed traces of ABA from the water. The conversion of the 1–4 allenic group into a 1′-hydroxy-4-ene is less surprising because liverworts contain the xanthophyll neoxanthin [9, 10], which has an allenic group at the homologous position. Presumably, the fronds are capable of degrading it as well. We have subsequently found that *Riccia* thalli do contain ABA so the original suggestion that it is not made by liverworts requires revision.

Yeast does not contain xanthophylls and did not form ABA from compounds **2**, **3**, **8** and **9**. The fungus *Cercospora rosicola* does biosynthesize ABA, but no labelled 2-*trans*-ABA was detected when the $^2H_2$ labelled allene **3** was supplied to the hyphal mat.

The racemic, labelled 2-*cis*[7′-$^2H_3$] allenic alcohol acetate **2** was converted into ABA in large quantities by non-sterile *Lemna* and *Riccia* fronds. Extracts from the medium and the plants were methylated and GC-MS analysis established that [$^2H_3$]Me ABA was present in both. The base peak of endogenous ABA at *m/z* 190 (Fig. 2) was accompanied by a nineteen-fold larger peak at *m/z* 193. This considerable excess of adventitiously derived ABA suggests that the enzymes which carry out the conversion of the analogues in *Lemna* and *Riccia* are not subject to the same regulatory controls as affect those that biosynthesize the endogenous hormone. The [$^2H_3$]Me ABA was separated into its (+)-*S* and (−)-*R* forms by HPLC in a Chiralcel column and equal amounts of labelled natural and unnatural enantiomers were present. The 7′-$^2H_3$ labelled acetylenic alcoholic analogue **8** was not converted into ABA but the acetylenic 7′-$^2H_3$ labelled acetylenic acid **9** did give traces of [$^2H_3$]ABA.

The growth inhibition produced by the analogues cannot be compared exactly and quantitatively with that engendered by ABA because, on the one hand, an initial application of ABA is immediately potent and the concentration within cells of *Dodonaea viscosa* in cell suspension cultures has been found to fall rapidly after 2 hr so that the concentration within the cells is little above the normal endogenous values [11]. On the other hand **2**, **3** and **4**, being non-ionic and less polar, probably enter the cells more rapidly than free ABA and are then converted into ABA, via several intermediates, over a longer term. AB alcohol has high growth inhibitory activity in several bioassays [12] and closes stomata [13]. It has been shown to be reversibly formed from AB aldehyde [14] which, in turn, is rapidly oxidized to ABA [14]. A more constant exposure to a high concentration of ABA could occur by this means which may be more effective as an inhibitor of growth than the feeding of a pulse of ABA. The greater growth inhibitory activity of xanthoxin [12] and a mixture of **6** and **7** in comparison with ABA [1] has already been documented. We cannot discount the possibility that the starting compounds or materials formed from them may also be able to interact at sites affected by ABA.

CONCLUSIONS

We have established unambiguously that the deuterium-labelled allenic analogue of AB alcohol acetate is converted into ABA by axenic *L. paucicostata* plants. The reactions and intermediates involved have not been identified, indeed, there may be no unique sequence of steps but rather several paths by which the molecules undergo permitted reactions. However, deuteriated AB aldehyde has been detected in the growth medium in quantities similar to those of deuteriated ABA, so it appears that the allenic group can be attacked before C-1 is oxidized to carboxylic acid. The oxygen atom added at C-1′ is derived from the medium, not from O_2. This discriminates between the conversion of the allenes into ABA and the endogenous biosynthesis. When a sample of the ABA fraction (90%) was methylated and resolved in a chiralcel HPLC column virtually identical amounts of (+)-[$^2H_3$]- and (−)-[$^2H_3$]Me ABA were measured. This suggests that epimerization of C-1′ occurs in an intermediate. Yeast and *Cercospora rosicola* lack the ability to convert the allene into ABA.

EXPERIMENTAL

Growth of plants. Axenic duckweed fronds (*Lemna gibba* and *L. paucicostata*) were grown under continuous light at 25° on the medium described in ref. [15]. A concentrate of major nutrients was prepared which gave the following mixture when diluted × 100: KH_2PO_4, 503; KNO_3, 889; $Ca(NO_3)_2$, 638; $MgSO_4$, 244 mg^{-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 Na_4EDTA and 3.3 mg $FeCl_3$ were added together with 1 ml of a microelement stock soln (prepared freshly every few weeks) which gave the following final concentrations in mg l^{-1} H_3BO_4, 2.9; $ZnSO_4$, 0.15; Na_2Mo_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 to 10 axenic fronds in 100 ml of sterile medium in a 250 ml conical flask stoppered with cotton wool and they grew for 8–12 days under continuous light before requiring subculturing.

In the earlier experiments the larger *L. gibba* was used, but *L. paucicostata* was inhibited by ABA to the same degree and grew more readily in small vols of culture medium and so was preferred for small scale metabolic experiments.

In addition, a growing culture of baker's yeast (*Saccaromyces cerevisiae*) was started by adding a commercial, dried preparation to a sucrose soln (5% at 30°). After 24 hr the solns containing the test compounds were added and incubated for 50 hr. The cells were removed by centrifugation and the supernatant and cell pellets analysed as before. A culture of *Cercospora rosicola* (strain 138.35, Central Bureau voor Schimmel Cultures, Baarn, Holland) was grown on potato dextrose agar in a petri dish for 7 days at 20° and when the colonies had almost coalesced the solution of an allene in EtOH–H_2O (7:3, 50 μl) was dropped onto the thallus.

The compounds were supplied to the *Lemna* plants (5 g fr. wt) by placing the fronds in sterile, 100 ml, glass flasks plus medium (20 ml) and stoppered with cotton wool. A measured quantity of each compound, dissolved in Me_2CO was injected into the growth flask to a final concentration of less than 1%. No contamination of the culture medium by bacteria or fungi occurred when these methods were used and no differences were observed between untreated controls and controls containing

blank Me_2CO at the same concns as the experimental solns.

Riccia fluitans, a liverwort that grows in water as a dense mat of floating thalli was obtained from an aquarium shop in Saskatoon. The plants were blotted and placed in aq. solns (10 ml) of the test compounds, 50 hr, room temp.

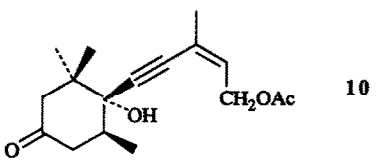
The conversion of the allene into ABA by fronds of *Lemna paucicostata* growing on $H_2{}^{18}O$ (98 atom %) was carried out by drying 0.5 ml medium in a sterile 2 ml Reactivial and then adding the $H_2{}^{18}O$ (0.5 ml) and the allene (10 μg in 5 μl, EtOH–H_2O, 7:3). The ABA was extracted, separately, from the fronds and the medium after 7 days growth in darkness, and the samples were converted into pentafluorobenzyl esters [6].

Extraction. The *Lemna* fronds were extracted in HOAc–Me_2CO (49:1) to which the antioxidant 2,6-di-*tert*.butyl-4-methyl phenol (BHT)) had been added (20 mg l^{-1}). The medium was acidified to pH 3 and then extracted with an equal vol of Et_2O ×3. The solvents were removed under a stream of N_2 and the residues taken up in a minimum vol of EtOH (0.3 ml), diluted with aq. HOAc (49:1, 20 ml) and passed slowly through a C18 reversed-phase Sep-pak filter (Waters-Millipore, Bedford, MA, U.S.A.). This was washed with EtOH–HOAc–H_2O (5:2:93; 10 ml) and then the ABA was eluted with EtOH–H_2O (3:7), dried, methylated with CH_2N_2 and subjected to NICI(CH_4)GC-MS or PIEIGC-MS as described earlier [1]. Et_2O extracts of the medium were also examined without methylation.

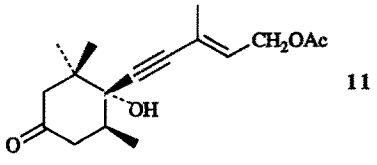
Synthesis of compounds. Isomerization of **10** with MeO^2H and ^2H_2O gave the 2-*cis* allenic acetate **3** with two ^2H atoms incorporated, one at the allenic C-4 position and the other on the ring vinyl position C-3′. The 2-*cis*- and 2-*trans*-isomers were sepd by HPLC and the deuteriated allene **7** prepared from the deconjugated acetylenic compound as for the *cis* series.

Saponification of the 2-*trans* ketomethyl ester **9** to the acid **12** in NaO^2H–^2H_2O and $C^2H_3O^2H$ was observed by NMR to be complete in less than 1 hr at ambient temp. Total exchange of the methylene and two vinyl protons occurred under the conditions of the reaction while the vinyl methyl protons were unaffected.

C-3′, C-4 *dideuteriated* 2-cis *allenic acetate.* To a soln of *cis* enynol **8** (30 mg. 0.1 mmol) in MeO^2H (5.0 ml) at 0° was added 5 mg solid K_2CO_3. After 20 min ^2H_2O was added to the red. soln and the 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 deuteriated *cis* allenic acetate 11 mg, 37% yield. ^1H NMR: δ6.67 (0.12H, *s*, H-4), 5.89 (0.08H, *m*, H-3′), 5.50 (1.0H, *t* further split, J=7.3 Hz, H-2) 4.69 (2.0H, *d*, J =7.3 Hz, H-1) 2.38 (2H, AB*q*, J=16Hz, H-5′), 2.06 (3H, *s*, OCMe), 1.96 (3H, *m*, Me-2′), 1.80 (3H, *m*, Me-3), 1.18 (3H, *s*, Me-6′), 1.17 (3H, *s*, Me-6′); IR ν_{max}^{film} cm^{-1}: 1900, 1720, 1650 and 1580; GC-MS 24 eV *m/z* (rel. int.): 276 (1), 234 (25), 215 (57) and 160 (100). This gave 92% ^2H at C-3′ and 88% ^2H at C-4.



10



11

C-3′, C-4 *dideuterated* 2-trans *allenic acetate.* A soln of *trans* enynol **11** (61 mg) was treated as above to afford deuteriated *trans* allenic acetate 22 mg (37%) which gave a single spot on TLC (R_f 0.3, Et_2O–hexane, 1:1); ^1H NMR (360 MHz, C^2HCl_3): δ6.31 (0.13H, *br s*, H-4), 5.89 (0.48H, *t*, J=1.1 Hz, H-3′), 5.61 (1H, *tq* J=7.0, 1.3 Hz, H-2), 4.68 (2.0H, *d*, J=7.0 Hz, H-1), 2.38 (2H, *br s*, H-5′), 2.06 (3H, *s*, OMe), 1.94 (3H, *d*, J=1.1 Hz, Me-2′), 1.75 (3H, *br s*, Me-3), 1.18 (3H, *s*, Me-6′) and 1.15 (3H, *s*, Me-6′); IR ν_{max}^{film} cm^{-1}: 1910, 1730, 1650 and 1590; GC-MS *m/z* 276 (5), 234 (6) and 215 (100). This gave 48% ^2H at C-3′ and 87% ^2H at C-4.

C-7′ *trideuterated* cis *allenic acetate.* Using the analogous procedure 2-*cis*[7′-$^2H_3$]allenic acetate **2** gave ^1H NMR: δ6.67 (1H, *s*, H-4), 5.89 (1H, *m*, H-3′), 5.50 (1H, *t* further split, J=7.3 Hz, H-2), 4.69 (2H, *d*, J=7.3 Hz, H-1), 2.38 (2H AB*q*, J=16 Hz, H-5′), 2.06 (3.0H, *s*, OMe), 1.96 (0.3H, *m*, Me-2′), 1.80 (3H, *m*, Me-3), 1.18 (3H, *s*, Me-6′), 1.17 (3H, *s*, Me-6′); MS (in beam EI) *m/z* (rel. int.): 277 (10), 235 (20) and 217 (100): non-deuteriated analogue gave *m/z* 274 (2), 232 (8) and 214 (100). The deuterium incorporation at C-7′ was 90%.

6,6-*Dimethyl-trideuteriomethyl*-3,5,5-*trideuterocyclohex*-2-*en*-1,4-*dione.* Acetyl chloride (200 ml) was added at a rate maintaining the int. temp. less than 30° to a stirred soln of ^2H_2O (600 ml) in a round bottomed flask externally cooled by a dry ice–ethylene glycol bath, at a rate maintaining the int. temp. less than 30°. The mixture was stirred at ambient temp. for 16 hr after the addition of oxoisophorone (25 g, 165 mol) in dioxane (100 ml) and then heated to reflux for an additional 6 hr. The cooled mixture was extracted with Et_2O (×4). The combined organic phases were stirred with satd $NaHCO_3$ soln and solid $NaHCO_3$ was added until no further bubbling was observed. The phases were sepd and the aq. phase was extracted with Et_2O. The combined ethereal extracts were washed successively with satd $NaHCO_3$ soln and then dried over Na_2SO_4. Evapn of the solvent and distillation afforded deuteriated oxoisophorone 20.85 g, bp 68° at 1.0 Torr in 80% yield [16]. The product gave ^1H NMR: δ6.5 (*s*, 0.03H, vinyl H), 2.7 (*s* 0.13H, CH_2), 1.9 (*m*, 0.20H, vinyl Me) and 1.21 (*s*, 6.0H, *gem* Me); IR ν_{max}^{film} cm^{-1}: 1680–1660 GC-EIMS *m/z* (rel. int.): 158 $[M]^+$ (30), 100 $[M \times {}^2H_2C{=}(Me)_2]^+$ (70), 72 $[M - {}^2H_2C{=}(Me)_2CO]^+$ (100), with relative intensities for the molecular ion cluster: 160 (9), 159 (15), 158 (100) and 157 (29) for a calcd isotope enrichment for $^2H_6$ 94–97%.

6,6-Dimethyl-2-trideuteriomethyl-cyclohexan-1,4-dione. Reduction of the deuteriated oxoisophorone (2.5 g) with baker's yeast was carried out according to the procedure of Lamb and Abrams affording 1.9 g of 6,6-dimethyl-2-trideuteriomethyl-3,5,5-trideuteriocyclohexan-1,4-dione that give GC-EIMS *m/z* 160 (21), 159 (13), 145 (27) and 58 (80). The crude crystalline product (1.0 g) was dissolved in MeOH (10 ml) and H_2O (10 ml) containing K_2CO_3 (250 mg) was added. After 3 hr, H_2O was added and the reaction mixture extracted with Et_2O (×3). The combined organic phases were washed with NaCl soln and dried over Na_2SO_4. Evapn of the solvent afforded 900 mg of product which was crystallized from Et_2O–hexane to give trideuterio compound 600 mg, mp 65.5–66.5°, GC-EIMS *m/z* (rel. int.): 157 $[M]^+$ (24), 142 $[M-Me]^+$ (35), 56 (82), with relative intensities for the molecular ion cluster: 160 (6), 159 (8), 158 (17), 157 (100) and 156 (9) for a calcd isotope enrichment for $^2H_3$ 108%, or 3.24 ^2H per molecule.

4-Hydroxy-4-(5-hydroxy-3-methylpent-3-en-1-ynyl)-3,3-dimethyl-5-trideuteriomethylcyclohexanone. The monoethyleneketal of 6,6-dimethyl-2-trideuteriomethyl-cyclohexan-1,4-dione was prepared by reaction of the dione (770 mg, 4.9 mmol) with ethyleneglycol (330 μl, 1.2 equivalents) with *p*-toluenesulphonic acid (10 mg) in C_6H_6 (5 ml) under reflux with a Dean Stark trap. After 3 hr, the soln was cooled, satd $NaHCO_3$ soln was added, and the product extracted with Et_2O (×3). The combined organic phases were washed with satd NaCl soln, and dried over Na_2SO_4. After evapn of the solvent, the crude product, a mixture of mono and bis ketal (930 mg) was alkylated with the dilithium salt of *cis*-3-methylpent-4-yn-2-en-1-ol (440 mg, 4.6 mmol) [17]. The crude product (1.34 g) in THF (25 ml) was treated with 10% HCl (25 ml) at ambient temp. for 2 hr. Satd $NaHCO_3$ was added and the product extracted with Et_2O (×3). The combined organic phases were washed with NaCl soln and dried over Na_2SO_4. Evapn of the solvent afforded 1:1 g crude product which was chromatographed over silica gel eluting with Et_2O–hexane (3:1). The purified product (344 mg) obtained in 30% overall yield, gave a single spot on TLC (R_f 0.25 in Et_2O–hexane, 3:1): ^1H NMR: δ5.92 (*ddq*, J=6.6, 6.6, 1.1 Hz, 1H =CH), 4.32 (*dq*, J=6.6, 0.9 Hz, 2H, CH_2O), 2.64 (*d*, J=14.3 Hz, 1H, H-5'eq), 2.29 (*m*, $W_{1/2}$, J=2 Hz, 3H, $CHCD_3$, H-3'eq and ax), 2.09 (*d*, J=14.3 Hz, 1H, H-5'ax), 1.91 (*d*, J=1.1 Hz, 3H, MeC=C), 1.20, 0.98 (2*s*, 6H, *gem* Me).

2-cis-Trideuteriomethyl allenic acetate. Using the same procedure as employed for the non-deuteriated analogue (**1**), the deuteriated cyclohexanone (128 mg, 0.5 mmol) was dehydrated and acetylated to afford the enynol (72 mg) which gave ^1H NMR: δ5.77 (*t*, J=6.9 Hz, 1H, H-2), 4.76 (*d*, J=6.9 Hz, 2H, CH_2O), 2.90 (*s*, 2H, H-3'), 2.40 (*s*, 2H, H-5'), 2.04 (*s*, 3H, MeCO), 1.95 (*s*, 3H, Me-3), 1.16 (*s*, 6H, *gem* Me); MS molecular ion *m/z* 277. Rearrangement of the enynacetate to the allenic compound was performed as previously described using 55 mg enynol with K_2CO_3 (5 mg) and MeOH (3 ml). The product after silica gel chromatography gave a single spot on TLC (R_f 0.4 in Et_2O–hexane, 1:1); ^1H NMR: δ6.67 (*s*, 1H, allenic H), 5.89 (*s*, 1H, H-3'), 5.51 (*t*, J=7.2 Hz, 1H, H-2), 4.69 (*d*, J=7.2 Hz, 2H, OCH_2), 2.38 (*m*, 2H, H-5'), 2.06 (*s*, 3H, OMe), 1.80 (*s*, 3H, Me-3), 1.18 and 1.17 (2*s*, 6H, *gem* Me); MS *m/z*: 277 (12), 235 (20) and 217 (100).

Mass spectra. Methane positive or negative ion CI spectra were obtained using the apparatus described previously [5]. The compounds were introduced by GC into a 15 m × 0.32 mm i.d. BP-5 column, film thickness 1 μm, carrier gas was He at 2 ml min^{-1} and temp. programmed from 150° at 10 min to 200°. PIEIGC mass spectra were obtained using a DB-5 column (60 m).

Acknowledgements—We thank Judy Le Claire for the work on axenic cultures and Nancy Lamb and Angela Shaw for expert assistance with the syntheses. In addition, we thank Dave Bourne, Doug Olson, Lawrence Hogge and Robert Willows for help with mass spectrometry and Ted Mazwek and Brock Chaston for NMR spectroscopy. This work was supported, in part, by the Australian Research Committee.

REFERENCES

1. Abrams, S. R. and Milborrow, B. V. (1991) *Phytochemistry* **30**, 3189.
2. Perry, T. O. and Byrne, O. R. (1969) *Plant Physiol.* **44**, 784.
3. Stewart, G. R. (1969) *Nature* **221**, 61.
4. Netting, A. G., Milborrow, B. V., Vaughan, G. T. and Lidgard, R. O. (1987) *Biomed. Environ. Mass Spectrom.* **15**, 375.
5. Netting, A. G. and Milborrow, B. V. (1988) *Biomed. Environ. Mass. Spectrom.* **17**, 281.
6. Heath, T. G., Gage, D. A., Zeevaart, J. A. D. and Watson, J. T. (1990) *Org. Mass Spec.* **25**, 655.
7. Zeevaart, J. A. D. and Milborrow, B. V. (1976) *Phytochemistry* **15**, 493.
8. Pryce, R. J. (1971) *Planta* **97**, 354.
9. Mues, R., Edelbluth, E. and Zinsmeister, H. D. (1973) *Osterr. Bot. Zeit.* **122**, 177.
10. Szewczyk, E. (1978) *Acta Biol. Cracov Ser. Bot.* **21**, 75.
11. Windsor, M. L., Milborrow, B. V. and McFarlane, I. J. (1992) *Plant Physiol.* **100**, 52.
12. Taylor, H. G., Burden, R. S. (1972) *Proc. R. Soc. B.* **180**, 317.
13. Orton, P. J. and Mansfield, T. A. (1974) *Planta* **121**, 263.
14. Linforth, R. S. T., Bowman, W. R., Griffin, D. A., Marples, B. A. and Taylor, I. B. (1987) *Plant Cell Environ.* **10**, 599.
15. Maeng, J. and Khudairi, A. K. (1973) *Physiol. Plant.* **28**, 264.
16. Murray, R. H. D., McCabe, P. H. and Hogg, T. C. (1969) *Tetrahedron* **25**, 5839.
17. Lamb, N. and Abrams, S. R. (1990) *Can. J. Chem.* **68**, 1151.