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THE METABOLISM OF α-IONYLIDENE COMPOUNDS BY *CERCOSPORA ROSICOLA**

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Key Word Index—*Cercospora rosicola*; abscisic acid; biosynthesis; 1′-hydroxy-α-ionylidene acetic acid; 4′-hydroxy-α-ionylidene acetic acid; α-ionylidene ethanol; stereospecificity.

Abstract—α-Ionylidene ethanol was converted to 4′hydroxy-α-ionylidene acetic acid, 1′-deoxy-ABA and ABA by *Cercospora rosicola*. Both the 4′-(*R*)- and 4′-(*S*)-epimers of 4′-hydroxy-α-ionylidene acetic acid were detected but the configuration of the 1′-position was not established. Both epimers were metabolized to 1′-deoxy-ABA and ABA. Both the *cis*- and *trans* 1′,4′diols of ABA were also converted to ABA. 1′-Deoxy-ABA was stereospecifically hydroxylated to form ABA. 1′-Hydroxy-α-ionylidene derivatives inhibited ABA production and were only oxidised to ABA in low yield. α-Ionylidene ethanol, α-ionylidene acetic acid and both epimers of 4′-hydroxy-α-ionylidene acetic acid were identified as endogenous compounds.

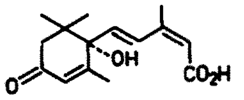
INTRODUCTION

In the fungus *Cercospora rosicola* α-ionylidene derivatives are converted to ABA (**1**) via the endogenous intermediate 1′-deoxy-ABA (**2**) [1, 2]. This pathway also operates in *C. cruenta* [3], although in this species the major biosynthetic route may involve oxidation of γ-ionylidene ethanol [4, 5]. In this paper we present further data relating to the oxidation of α-ionylidene derivatives by *C. rosicola* and identify α-ionylidene ethanol (**4**) as an endogenous compound.

RESULTS AND DISCUSSION

α-[$^2H_3$]Ionylidene ethanol (**4**) was rapidly and extensively metabolized by resuspensions of *C. rosicola* that were actively synthesizing ABA (Table 1). After *ca* 20 hr no residual **4** was detectable and 35% of the applied compound had been converted to 1′-deoxy-ABA (**2**) and ABA. The two 4′-epimers of 4′-hydroxy-α-ionylidene acetic acid (**5**, **6**) were also identified as metabolites on the basis of high pressure- and gas liquid chromatography retention times and GC/MS characteristics. Neither compound was diluted with unlabelled material. The stereochemistry of the 1′-position was not determined. Both epimers were also detected as metabolites of α-[$^2H_3$]ionylidene acetic acid (**3**). In all cases the (*R*)-epimer (**5**) was the major (60–80%) and the (*S*)-epimer (**6**) the minor component. Ichimura *et al.* [3] and Norman *et al.* [2] have also identified 4′-hydroxy-α-ionylidene acetic acid as a metabolite of labelled **3** in *C. cruenta* and *C. rosicola* respectively. In *C. cruenta* the (*S*)-epimer was the predominant form, contrary to the results presented here. In addition, both epimers were found to have the (*S*)-configuration at the 1′-position, suggesting that further metabolism of the 4′-hydroxy- compounds is stereoselective [3].

Both epimers of 4′-hydroxy-α-ionylidene acetic acid (**5**, **6**) were converted to ABA and 1′-deoxy-ABA when applied to primary cultures of *C. rosicola* (Table 2). Both the 1′4′ *cis*- and *trans*-diols of ABA (**7**, **8**) were also converted to ABA, demonstrating that the required enzymes do not exhibit a high degree of substrate specificity.



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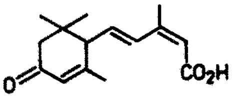

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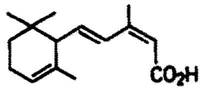

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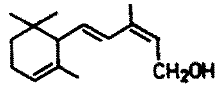

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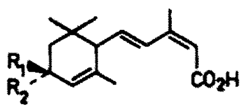

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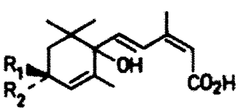
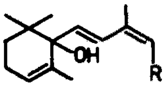
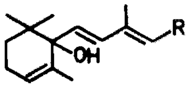
7 R_1=OH,R_2=H



6 R_1=H,R_2=OH
8 R_1=OH,R_2=H



9 R=CH_2OH
11 R=CO_2H



10 R=CH_2OH
12 R=CO_2H

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*Part 4 in the series 'Biosynthesis of ABA in *C. rosicola*'. For part 3 see ref. [10].

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Table 1. Metabolism of 2-[^{2}H/^{3}H] ionylidene ethanol (**4**) by *C. rosicola*

	Radioactivity (% dose)		Specific incorporation
	(dpm × 10^{-4})	(% distribution)	
Acid Et_2O	140		74.5
Basic Et_2O	26		13.8
Aqueous	9		4.8
Mycelium	13		6.9
(*R*)-4'-Hydroxy-α-ionylidene acetic acid (**5**)	8.7	4.6	100
(*S*)-4'Hydroxy-α-ionylidene acetic acid (**6**)	5.3	2.8	100
1'-Deoxy-ABA (**2**)	28	15	65
ABA (**1**)	35	19*	60

*Corrected for extraction losses.

Table 2. Incorporation of [^{2}H/^{3}H]-4'hydroxy-α-ionylidene acetic acids (**5**, **6**) and [^{3}H] *cis* and *trans*-ABA-diols (**7**, **8**) into 1'-deoxy-ABA (**2**) and ABA (**1**) by primary cultures of *C. rosicola*

	1'-Deoxy-ABA		
	(% dose)	(specific incorporation)	(% dose)
(*R*)-4'-Hydroxy-α-ionylidene acetic acid (**5**)	3.3	40	
(*S*)-4'Hydroxy-α-ionylidene acetic acid (**6**)	3.4	82	
cis-ABA-diol (**7**)	—		17*
trans-ABA-diol (**8**)	—		18**

*4% and ** 0% incorporation by boiled controls.

1'-Deoxy-[^{2}H] ABA (**2**) was stereospecifically converted to ABA by a resuspension of *C. rosicola*. The ORD spectrum of ABA extracted from cultures to which **2** had been applied was identical to that of ABA extracted from control cultures. ORD analysis of residual [^{2}H]-**2** extracted from fed cultures revealed that it had the opposite configuration to **2** extracted from control cultures (Fig. 1). Thus the 1'-hydroxylase enzyme is stereospecific with regard to the configuration at C-1' in addition to the geometry of the side-chain [1].

Both geometric isomers of 1'-hydroxy-α-ionylidene ethanol (**9**, **11**) and 1'-hydroxy-α-ionylidene acetic acid (**10**, **12**) inhibited ABA production when applied to resuspensions of *C. rosicola* (Fig. 2), although ABA production recommenced after *ca* 24 hr in cultures fed **9**. Very low incorporation into ABA or 2-*trans*-ABA was observed (Table 3). Such low incorporation suggests that the 1'-hydroxy compounds are poor substrates for the enzymes normally responsible for oxidation of α-ionylidene derivatives (compare Table 3 with Table 1) and not, therefore, endogenous intermediates. Norman *et al.* [2] reported that small amounts of **10** were formed from applied α-ionylidene acetic acid (**3**). Although we have not identified **10** following application of [^{2}H]-**3** or **4**, several labelled compounds with the basic α-ionylidene structure are routinely detected as minor metabolites, as evidenced by the presence of characteristic ions at *m/z* 136 and 128 in GC/MS analyses. Norman *et al.* [2] applied the ethyl esters of **10** and **12** to *C. rosicola* resuspensions and observed substantial conversion to ethyl-ABA and ethyl-*trans*-ABA. Small amounts of ethyl-1',4'-*trans*-ABA diol

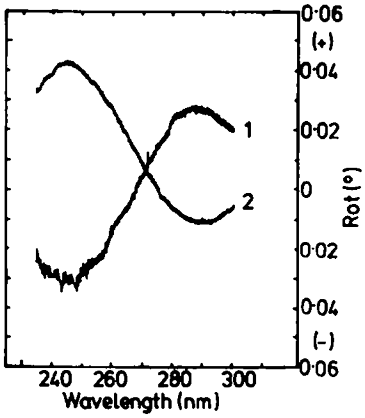

Fig. 1. ORD spectrum of methylated 1'-deoxy-ABA (**2**) extracted from *C. rosicola* cultures. 1: 1'-deoxy-ABA extracted from control flasks; 2: residual 1'-deoxy-ABA extracted after feed of (*R*, *S*)-1'-deoxy-ABA.

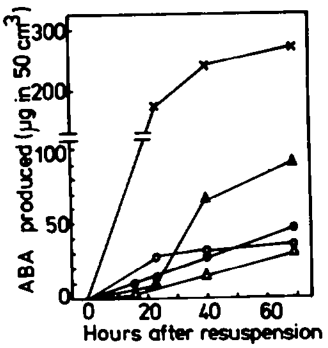

Fig. 2. Inhibition of ABA production in *C. rosicola* resuspensions by 1′-hydroxy-ionylidene derivatives. X,–X, control; ▲——▲, 9; △——△, 10; ●——●, 11; ○—○, 12.

were also detected. High pressure liquid chromography analysis (system D) of cultures fed [^{2}H]-**10** revealed the presence of 1′,4′-*trans*-ABA diol (**8**). Gas chromatography/MS analysis confirmed its identity and showed that **8** was not diluted with unlabelled material. Both the *cis*- and *trans* 1′,4′-diols of ABA (**7**, **8**) can be converted to ABA by plant tissue [6] and the *trans*-diol (**8**) is also found as a metabolite of exogenous ABA in pea seedlings [6]. In addition, both diols have been reported as naturally occurring compounds [7, 8]. However, no trace of endogenous **8** was found in *Cercospora* culture filtrates. Hirai *et al.* [9] have recently presented evidence that 1′,4′-*trans*-ABA-diol (**8**) is an endogenous precursor of ABA in the fungus *Botrytis cinerea*. 1′-Deoxy-ABA (**2**) has also been detected in *B. cinerea* culture filtrates (Neill and Horgan, unpublished).

α-Ionylidene ethanol (**4**) was identified by GC/MS as an endogenous constituent of mycelium harvested just at the beginning of the phase of rapid ABA synthesis. The levels of **4** were extremely low, *ca* 500 ng being recovered from the extract of *ca* 80 g wet weight of mycelium. Exogenous **4** was metabolized very rapidly by mycelium (Table 1).

α-Ionylidene acetic acid (**3**) was tentatively identified in mycelial extracts on the basis of HPLC and GLC retention times and by GC/MS with selected ion monitoring. A single peak co-chromatographed with authentic methyl-**3** when ions at the *m/z* values 248, 133 and 125 were monitored. It was not possible to obtain a full MS due to contamination with fatty acids. Endogenous **3** was present at very low concentrations and exogenous **3** rapidly metabolized.

The two 4′-epimers of 4′-hydroxy-α-ionylidene acetic acid (**5**, **6**) were identified as endogenous components of filtrates of cultures in which ABA biosynthesis had been inhibited following application of dehydrofarnesol [10]. Identity was established on the basis of HPLC and GLC retention times and MS characteristics: **5** (methyl-ester): 264 [M]$^+$ (1), 246 (1), 221 (3), 125 (100), 122 (71), 107 (41); **6** (methyl-ester): 177 (5), 171 (8), 161 (10), 133 (25), 125 (100), 112 (15), 111 (18). Further evidence for the presence of **6** was obtained following a feed of [^{2}H]-**3** in which the mass spectrum of isolated **6** revealed clearly that the deuterium label had been diluted with endogenous material.

The identification of **2**–**6** as naturally occurring compounds, the ease of their conversion into ABA and the poor incorporation of 1′-hydroxy-α-ionylidene derivatives provide strong evidence that the pathway of ABA biosynthesis in *Cercospora rosicola* involves oxidation of α-ionylidene ethanol, 1′-hydroxylation being the final step.

EXPERIMENTAL

Materials. *Cercospora rosicola* Passerini was maintained and sub-cultured as described previously [10]. Large-scale batch cultures were grown in 4 l medium contained in 5 l pots. The pots were continuously stirred and sparged with filter-sterilized air.

Administration of labelled compounds. [^{2}H/^{3}H]-**5** and **6** (*ca* 0.3 mg, 4 × 10^5 dpm) and [^{3}H]-**7** and **8** (*ca* 0.3 mg, 5 × 10^5 dpm) were applied to primary cultures (50 ml) in 200 μl EtOH as described previously [1]. [^{2}H/^{3}H]-**4** (1.4 mg, 1.9 × 10^6 dpm) and [^{2}H]-**2** (1 mg) were applied to 100 ml resuspensions and 0.6 mg of the [^{2}H]-1-hydroxy-α-ionylidene derivatives (**9**–**12**) to 50 ml as described in the preceding paper [10]. Cultures were usually harvested after 24 hr. ABA concentrations were determined as described previously [1].

Chromatography. HPLC was performed as described previously. HPLC systems: A: ODS-Spherisorb-2 (150 × 10 mm i.d.), eluted with a linear gradient of 20–100% MeOH in 0.1 M HOAc over 40 min, 5 ml/min; B: ODS-Spherisorb-2 (150 × 10 mm i.d.), eluted with a linear gradient of 50–100% MeOH in 0.1 M HOAc over 30 min, 5 ml/min; C: ODS-Spherisorb-2 (150 × 10 mm i.d.), eluted with 75% MeOH–0.1 M HOAc, 5 ml/min; D: ODS-Spherisorb (150 × 4.5 mm i.d.) eluted with 40% MeOH–0.1 M HoAC, 2 ml/min; E: Spherisorb (250 × 4.5 mm i.d.), eluted with hexane–*iso*-PrOH (100:1.25), 2 ml/min; F: Spherisorb (250 × 4.5 mm i.d.), eluted with hexane–$CHCl_3$–*iso*-PrOH (9:5:2) 2 ml/min; G: Partisil-PAC (150 × 10 mm i.d.), eluted with hexane-EtOH (100:1:5), 5 ml/min.

MS. GC/MS: Kratos MS 25/DS 55C linked to a Carlo Erba 4200 GC. GC: BP-1 (12 m × 0.33 mm i.d.) or Mega-OV-1 (15 m × 0.32 mm i.d.) capillary column, He 5000 kg/m^2, Grob splitless injector 260°, column 150 or 230° increasing at 8°/min. MS: source 190°, jet separator 230°, 70 eV, scan rate 1 sec/decade.

Table 3. Incorporation of [^{2}H]-1′-hydroxy-α-ionylidene derivatives into ABA by *C. rosicola*

	ABA		2-*trans*-ABA	
	(% dose)	(specific incorporation)	(% dose)	(specific incorporation)
1′-Hydroxy-α-ionylidene ethanol (**9**)	1	0.07	—	—
1′-Hydroxy-α-ionylidene acetic acid (**10**)	2.5	31	—	—
2-*trans*-1′-Hydroxy-α-ethanol (**11**)	0	—	1.5	89
2-*trans*-1′Hydroxy-α-ionylidene acetic acid (**12**)	0	—	0.8	93

Multiple ion monitoring was performed under computer control with a dwell time of 140 msec, a settling time of 100 msec and a 50 μsec sample period.

NMR. 90 MHz Perkin-Elmer; 360 MHz Bruker spectrospin. *ORD.* ORD spectra were obtained on a JASCO spectropolarimeter.

Extraction and purification of metabolites. Basic and acid Et_2O fractions were prepared from the medium. 1'-Deoxy-ABA (**2**) and ABA (**1**) were purified by HPLC for GC/MS as described previously [1]. Percentage incorporation of labelled compounds was assessed by scintillation counting and GC/MS [1]. **5** and **6** were purified by HPLC on system A followed by methylation and HPLC on system E.

Identification of **3** *and* **4**. 84 g fr. wt of mycelium harvested from several large-scale cultures was extracted by homogenization in Me_2CO to yield 2.62 g of a brown tar. This was dissolved in 500 ml Et_2O and extracted with 500 ml 0.1 M HOAc. The Et_2O phase was evaporated to yield 0.84 g of a yellow oil, one-fifth of which was then dissolved in 0.4 ml MeOH plus 0.4 ml 0.1 M HOAc followed by centrifugation to yield a yellow/brown supernatant. This was fractionated by HPLC on system B and a large fraction corresponding to the R_t of **3** and **4** collected. This was chromatographed using system C and **3** and **4** collected separately. **4** was finally purified on system E prior to analysis by GC/MS. **3** was purified using system F and methylated with ethereal CH_2NH_2 prior to GC/MS. Methylated **3** was volatile and co-evaporated with solvent. ^{2}H-Labelled compounds were used as chromatography standards to avoid any possible problems of contamination.

Synthesis of (R)- *and* (S)-*4'-hydroxy-α-ionylidene acetic acids* (**5** *and* **6**). 2 mg of **2** was dissolved in 0.75 ml $MeOH–H_2O$ (2:1). A few crystals of $NaBH_4$ were added on ice and the reaction stopped after 1.5 hr by the addition of 1.5 ml Me_2CO. The reaction mixture was evapd to dryness and **5** and **6** purified by HPLC on system A. Portions of **5** and **6** were methylated and examined by GC/MS.

[^{1}H]-**5**: EIMS (GC/MS) *m/z* (rel. int.): 246 (6), 199 (8), 125 (100), 122 (50), 107 (30); [^{1}H]-**6**: EIMS (GC/MS), *m/z* (rel. int): 264 (1), 246 (8), 199 (12), 177 (4), 171 (10), 161 (6), 133 (11), 125 (100).

Two mg of methyl-**2** was reduced as above and Me-**5** and Me-**6** purified by HPLC on system G prior to 360 MHz ^{1}H NMR analysis. The configuration of the 4'-hydroxy group was determined from the half height width of the resonance of the 4'-proton in the 360 MHz spectrum, using the Karplus relationship to estimate coupling to adjacent protons. Me-**5** was assigned the (*R*) (eq.) configuration for the 4'-hydroxy group on the basis of a width at half height of 27 Hz. Me-**6** was assigned the (*S*) (ax.) configuration on the basis of a width at half height of 14 Hz. Peak widths were determined after OH decoupling.

2 mg of [^{2}H]-**2** + *ca* 1.5×10^6 dpm [^{3}H]-**2** (8.3 mCi/mmol [11]) were reduced as above and [^{2}H]-**5** and **6** purified on HPLC system A.

[^{2}H]-**5**: EIMS (GC/MS) *m/z* (rel. int.): 249 (6), 202 (8), 128 (100), 122 (60), 107 (40); [^{2}H]-**6**: EIMS (GC/MS), *m/z* (rel. int.): 267 (0.5), 249 (9), 202 (9), 180 (6), 174 (10), 162 (10), 128 (100).

1',4'-cis *and* trans *ABA-diols* (**7** *and* **8**). 0.5 mg of ABA and *ca.* 2×10^6 dpm [^{3}H]-ABA (20 mCi/mmol [12]) were reduced with $NaBH_4$ as above and [^{3}H]-**7** and **8** purified by HPLC on system A.

[^{1}H]-**7**: EIMS (GC/MS), *m/z* (rel. int.): 280 (0.5), 262 (10), 244 (6), 230 (10), 206 (25), 146 (32), 133 (25), 125 (100), 119 (45), 111 (25); [^{1}H]-**8** EIMS (GC/MS) *m/z* (rel. int): 262 (10), 244 (10), 230 (10), 206 (8), 146 (28), 133 (17), 125 (100), 119 (30), 111 (20).

1-Hydroxy-*α-ionone*. 2.5 g (22.5 mmol) freshly sublimed SeO_2 was dissolved in 300 ml 95% EtOH. This soln was added over 20 min to a stirred, refluxing soln of 5 g (26.0 mmol) α-ionone in 250 ml of 95% EtOH. The soln was stirred under reflux for a further 24 hr. After cooling, the soln was filtered through a Celite pad and rotary evaporated to a dark brown oil. The oil was 'flash chromatographed' on a 5 × 25 cm column of silica gel (Merck 9385) using hexane–EtOAc (12:3) as eluant. 50 ml fractions were collected. GLC and TLC analysis revealed that fractions 17–22 contained pure 1-hydroxy-α-ionone. The relevant fractions were reduced to dryness to yield 3.5 g of a light brown semi-solid material which was recrystallized from petrol to yield pure 1-hydroxy-α-ionone (3.1 g) as pale yellow crystals, mp 87–89° (lit. 89–90). The ^{1}H NMR spectrum of this material was identical to that published by Findlay and MacKay [13].

[$^2H_3$]-1-*Hydroxy-α-ionone*. 1-Hydroxy-α-ionone (1 g) was dissolved in 15 ml dry dioxane and 5 ml of ^2H_2O (99.8 atom % ^{2}H) was added. After the addition of 30 mg of metallic Na the mixture was heated to boiling and allowed to cool to room temp. before being rotary evaporated to a viscous oil. The above procedure was repeated twice. After the final evaporation step the oil was poured into 200 ml Et_2O. The Et_2O soln was washed × 4 with equal vols of H_2O before drying overnight with anhydrous $MgSO_4$. The Et_2O was removed and the residue recrystallized once from petrol to yield 640 mg [$^2H_3$]-1-hydroxy-α-ionone. The position and extent of deuteration was confirmed by mass spectrometry which showed only a $[M]^+$ of *m/z* 211 (100% $^2H_3$) and by the disappearance of the singlet at 2.25 in the ^{1}H NMR spectrum.

Methyl-1'-hydroxy-α-ionylidene acetates. An intimate mixture of 1-hydroxy-α-ionone (1 g, 4.8 mmol) and triphenylcarboxymethylene phosphorane (2 g, 5.9 mmol) was heated in an oil bath under N_2 at 180° for 1.5 hr. After cooling, the dark brown residue was suspended in 20 ml Et_2O and allowed to stand at *ca* 20° for 2 days. The resulting solid material was filtered off and washed with 20 ml Et_2O. The combined filtrates were reduced to dryness and the resulting brown oil flash chromatographed on a 30 × 5 cm column of silica gel (Merck 9385) using toluene–EtOAc (15:1) as the eluant. 50 ml fractions were collected. GLC examination of the fractions indicated the presence of single components in fractions 10–15 and 19–27. Removal of solvent gave 230 mg of the earlier eluting component and 160 mg of the later eluting component. The earlier eluting component was identified as methyl-2-*trans*-1'-hydroxy-α-ionylidene acetate on the basis of its ^{1}H NMR spectrum. The considerable deshielding of the proton on C-4 caused by the proximity of the carboxylic ester group when in the *trans* configuration was evidenced by the presence of a doublet (1H) at 7.65. The second component was identified as the 2-*cis*-isomer by virtue of this absorption occurring at 6.2.

The individual 2-*cis* and 2-*trans* methyl esters were hydrolysed to the free acids with methanolic KOH and reduced to the alcohols with $LiAlH_4$ in Et_2O. Portions of the alcohols and methylated acids were analysed by GC/MS. MS data: [^{1}H]-**9**: EIMS (GC/MS), *m/z* (rel. int.): 218 (30), 201 (5), 200 (5), 185 (11), 162 (100), 147 (45), 134 (25), 133 (14), 122 (40), 121 (32), 119 (61), 107 (82), 106 (20), 105 (30), 95 (15), 93 (20), 91 (42).

[^{1}H]-**11**: EIMS (GC/MS) *m/z* (rel. int.): 218 (40), 201 (12), 200 (20), 185 (32), 170 (52), 147 (38), 134 (32), 133 (21), 122 (22), 121 (26), 119 (100), 107 (10), 106 (22), 105 (48), 95 (14), 93 (22), 91 (56).

[^{1}H]-**10**: EIMS (GC/MS), *m/z* (rel. int.): 264 (21), 232 (18), 208 (15), 204 (15), 190 (10), 161 (29), 149 (32), 148 (100), 147 (25), 133 (47), 125 (36), 121 (23), 120 (12), 119 (14), 109 (15), 107 (14), 105 (25), 95 (20), 93 (15), 91 (25).

[^{1}H]-**12**: EIMS (GC/MS), *m/z* (rel. int.): 264 (12), 232 (12), 208 (11), 204 (8), 190 (8), 176 (51), 161 (20), 149 (100), 148 (75), 147 (18), 133 (32), 125 (18), 121 (8), 120 (12), 119 (11), 109 (12), 107 (11), 105 (20), 95 (16), 93 (15), 91 (20).

Methyl[$^2H_3$]-*1'-hydroxy-α-ionylidene acetates.* The 2-*cis* and

2-*trans* isomers of this compound were synthesized from $[^2H_3]$-1′-hydroxy-α-ionone by the method described above. Some loss of ^2H occurred in the course of the Witting reaction. The final product was shown by mass spectrometry to consist of 77% $[^2H_3]$, 20% $[^2H_2]$ and 3% $[^2H_1]$.

The 2-*cis* and 2-*trans* $[^2H]$-labelled 1′-hydroxy-α-ionylidene acetic acids and the corresponding alcohols were prepared from the esters by the standard methods. They were used for metabolic experiments without further purification. Portions of the $[^2H]$-alcohols and methylated acids were analysed by GC/MS.

$[^2H]$-**9**: EIMS (GC/MS) *m/z* (rel. int.): 221 (40), 220 (15), 204 (10), 203 (8), 188 (13), 165 (100), 164 (55), 150 (42), 149 (30), 137 (31), 136 (30), 135 (27), 122 (90), 121 (50), 120 (28), 119 (52), 109 (48), 108 (50), 107 (90), 106 (18), 105 (34), 95 (25), 93 (35), 91 (38).

$[^2H]$-**11**: EIMS (GC/MS) *m/z* (rel. int.): 221 (48), 220 (12), 204 (15), 203 (22), 188 (23), 165 (62), 164 (58), 150 (45), 149 (18), 137 (48), 136 (42), 135 (23), 122 (100), 121 (70), 120 (36), 119 (96), 110 (72), 109 (72), 18 (50), 107 (69), 106 (21), 105 (46), 95 (48), 93 (42), 91 (52).

$[^2H]$-**10**: EIMS (GC/MS) *m/z* (rel. int.): 267 (22), 266 (6), 235 (20), 234 (10), 211 (12), 207 (18), 202 (22), 190 (15), 179 (65), 178 (31), 164 (22), 152 (48), 151 (100), 150 (53), 149 (25), 136 (32), 128 (48), 124 (26), 123 (30), 109 (30), 108 (26), 107 (20), 105 (22), 95 (26), 93 (20), 91 (26).

$[^2H]$-**12**: EIMS (GC/MS) *m/z* (rel. int.): 267 (21), 235 (22), 211 (18), 207 (12), 202 (22), 179 (70), 178 (40), 164 (18), 152 (41), 151 (100), 150 (60), 149 (30), 136 (30), 133 (32), 128 (25), 124 (15), 123 (21), 109 (22), 108 (15), 107 (15), 105 (18), 95 (20), 93 (15), 91 (20).

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REFERENCES

1. Neill, S. J. and Horgan, R. (1983) *Phytochemistry* **22**, 2469.
2. Norman, S. M., Poling, S. M., Maier, V. P. and Nelson, M. D. (1985) *Agric. Biol. Chem.* **49**, 2317.
3. Ichimura, M., Oritani, T. and Yamashita, K. (1983) *Agric. Biol. Chem.* **47**, 1895.
4. Oritani, T., Niitsui, M., Kato, T. and Yamashita, K. (1985) *Agric. Biol. Chem.* **49**, 2819.
5. Oritani, T. and Yamashita, K. (1985) *Agric. Biol. Chem.* **49**, 245.
6. Milborrow, B. V. (1983) *J. Exp. Botany* **34**, 303.
7. Dathe, W. and Sembder, G. (1982) *Phytochemistry* **21**, 1798.
8. Milborrow, B. V. (1985) *Abstracts 12th International Conference Plant Growth Substances* (Bopp. M., Knoop, B. and Rademacher, W., eds) p. 28.
9. Hirai, N., Okamoto, M. and Koshimizu, K. (1985) *Abstr. 12th Int. Conf. Plant Growth Substances* (Bopp, M., Knoop, B. and Rademacher, W., eds) p. 30.
10. Neill, S. J., Horgan, R., Mercer, C. M. A. and Walton, D. C. (1987) *Phytochemistry* (submitted).
11. Neill, S. J., Horgan, R., Walton, D. C. and Lee, T. S. (1982) *Phytochemistry* **21**, 61.
12. Walton, D. C., Wellner, R. and Horgan, R. (1977) *Phytochemistry* **16**, 1059.
13. Findlay, J. A. and Mackay, W. D. (1971) *Can. J. Chem.* **49**, 2369.