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OCCURRENCE AND METABOLISM OF 1′,4′-*TRANS*-DIOL OF ABSCISIC ACID

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Key Word Index—*Pisum sativum*; pea; *Persea americana*; avocado; biosynthesis; abscisic acid; 1′,4′-*trans*-diol of abscisic acid; 1′,4′-dihydroxy-α-ionylideneacetic acid; 1′-*S*,4′-*S*-4′dihydroabscisic acid.

Abstract—The 1′,4′-*trans*-diol of abscisic acid was first identified in higher plants with GC-ECD and GC-SIM. The ^{2}H-labelled derivative was converted into abscisic acid (ABA) in pea and avocado, but ^{2}H-labelled ABA was not converted into the diol. These results suggest that the diol is one of the precursors of ABA in higher plants.

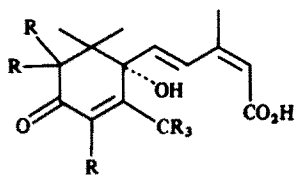
INTRODUCTION

Since Assante *et al.* [1] first reported abscisic acid (ABA) production by *Cercospora rosicola*, many phytopathogenic fungi that produce (+)-ABA (**1**) have been found [1–4]. Precursors of ABA have been isolated from *Cercospora* species [2, 5–7], but their natural occurrence in higher plants has not been reported. We have studied the metabolites of *Botrytis cinerea*, and isolated (+)-1′,4′-*trans*-diol of ABA (1′,4′-*t*-diolABA, **3**) from this fungus, where the compound was the immediate precursor of ABA [8]. We searched for 1′,4′-*t*-diolABA in higher plants using GC-ECD and selected ion monitoring (GC-SIM) in GC/mass spectrometry. This paper describes its identification and conversion into ABA in pea and avocado.

RESULTS AND DISCUSSION

Pea seedlings, avocado fruits, tomato fruits and banana pulp were selected to investigate the distribution of 1′,4′-*t*-diolABA. The EtOAc-soluble parts obtained from the plants were chromatographed on silica gel. For avocado, tomato and banana, 50–60% EtOAc eluates were analysed by GC-ECD after methylation. The small peak was eluted at the same retention time as the peak of 1′,4′-*t*-diolABA methyl ester in the chromatograms of avocado and tomato fruits. For pea seedlings, a fraction purified further was analysed by GC-ECD, and the result of analysis also suggested the presence of 1′,4′-*t*-diolABA. GC-SIM analysis was used for the eluates to identify 1′,4′-*t*-diolABA more precisely. Fragment ions at *m/z* 262 $[M-H_2O]^+$, 244 $[M-2H_2O]^+$ and 230 $[M-H_2O-MeOH]^+$, which are characteristic of 1′4′-*t*-diolABA methyl ester, were observed at the same retention time and relative intensity as those of standard 1′,4′-*t*-diolABA methyl ester in the chromatograms of pea and avocado. 1′-4′-*t*-DiolABA was not detected in tomato fruits by GC-SIM analysis, probably because of the detection limit. The endogenous concentration of 1′-4′-*t*-diolABA was 34.9 μg/100 g fr. wt for avocado and 137 ng/100 g fr. wt for pea, estimated by GC-SIM with (+)-[$^2H_7$]-1′,4′-*t*-diolABA (**4**) as the internal standard. 1′,4′-*cis*-DiolABA (**5**) was not detected in all of these plants.

1′,4′-*t*-DiolABA fed to wheat or bean is converted into ABA [9–12]. Therefore, 1′4′-*t*-diolABA in pea and avocado may be a precursor of ABA or a metabolite converted from ABA. To examine these possibilities, feeding experiments with ^{2}H-labelled compounds were done. (+)-[$^2H_7$]-1′,4′-*t*-DiolABA was fed to cut shoots of pea seedlings and extracted. After methylation, incorporation of the label was measured by GC/MS. The mass spectrum of ABA and phaseic acid (PA) methyl esters after administration of (+)-[$^2H_7$]-1′,4′-*t*-diolABA showed ions 4, 5 and 6 amu heavier than each molecular ion. The presence of these ions showed that the ^{2}H-labelled compound was incorporated into the ABA and PA. The ^{2}H-incorporation was measured by monitoring of the ions between *m/z* 278 and 284 for the ABA methyl ester and between *m/z* 294 and 300 for the PA methyl ester. The percentage of biosynthesized ABA and PA synthesized from (+)-[$^2H_7$]-1′,4′-*t*-diolABA was 95 and 76%, respectively. Incorporation of the label after administration of (+)-[$^2H_6$] ABA (**2**) was detected only in PA, and the percentage of biosynthesized PA synthesized from (+)-[$^2H_6$] ABA was 76%. GC/mass spectral analysis of 1′,4′-*t*-diolABA was unsuccessful because of the low concentration. Milborrow has reported that 8% of (±)-[2-^{14}C] ABA fed to pea seedlings is converted into 1′,4′-*t*-diolABA [11]. If 8% of the (+)-[$^2H_6$] ABA added is converted into 1′,4′-*t*-diolABA, 300 μg of the diol should be detected in our experimental conditions. However, the concentration of the diol after administration of (+)-[$^2H_6$]ABA was equal to the endogenous level. This suggested that (+)-[$^2H_6$]ABA was not converted into 1′,4′-*t*-diolABA in our experiment. This discrepancy might be caused by 1′,4′-*t*-diolABA being derived from (−)-ABA, or (+)-[$^2H_6$]ABA not being reducable because of the steric hindrance and isotopic effects of the deuterium atoms. To examine these possibilities, (±)-ABA was fed to pea seedlings, but the concentration of 1′,4′-*t*-diolABA did not increase. The pea seedlings used presumably have weak or no enzyme activity for the conversion of ABA into 1′,4′-*t*-diolABA. Little or no trace of 1′,4′-*t*-diolABA has been observed in autoradiograms of plant extracts after administration of radioactive (±)-ABA [12–15]. The equilibrium of the reaction in plants seems to favour



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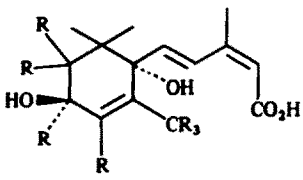
2 R = D



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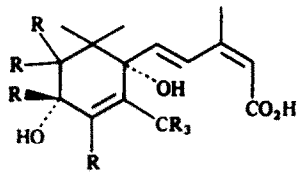

5 R = H
6 R = D

oxidation rather than reduction. (+)-[$^2H_7$]-1′,4′-*cis*-DiolABA (**6**) as well as (+)-[$^2H_7$]-1′,4′-*t*-diolABA was converted into ABA and PA by pea seedlings. The percentage of biosynthesized ABA and PA synthesized from (+)-[$^2H_7$]-1′,4′-*cis*-diolABA was 80 and 83%, respectively. These conversions presumably involve enzymatic oxidation of the diols, because the diols are stable in aqueous solutions of pH 2.5–7.5 for 10 days [8].

Avocado fruits also converted 1′,4′-*cis*- and *t*- diolABA into ABA, but ABA was not converted into 1′,4′-diolABAs. The percentage of biosynthesized ABA from (+)-[$^2H_7$]-1′,4′-*t*-diolABA was 14%, and that from (+)-[$^2H_7$]-1′,4′-*cis*-diolABA was 13%.

The above results show that 1′,4′-*t*-diolABA occurs naturally and that it is converted into ABA in higher plants. Thus, these experiments suggest that a biosynthetic pathway similar to that proposed for *B. cinerea* operates in higher plants. Although 1′,4′-*cis*-diolABA was not be detected as an endogenous compound, it has been identified in the seeds of *Vicia faba* [16]. The variety of 4′-hydroxylating enzymes may be caused by differences in the species, the developmental stage or both.

EXPERIMENTAL

Labelled compounds. The (+)-[$^2H_7$]-1′,4′-*cis*-and *t*-diolABA and the (+)-[$^2H_6$] ABA were synthesized as described previously [8].

Plants. Pea (*Pisum sativum* var. Alaska), avocado (*Persea americana*), banana (*Musa sapientum*) and tomato (*Lycopersicon esculentum*) were bought from a local supplier.

Natural occurrence of 1′,4′-t-diolABA in higher plants. Pea seedlings (0.385 kg), avocado fruits (0.465 kg), banana pulp (1.6 kg) and tomato fruits (2.1 kg) were extracted with MeOH. The extracts were defatted with *n*-hexane and then the EtOAc-soluble acidic parts were obtained in the usual way. These were chromatographed on silica gel with a mixture of EtOAc and toluene containing 1% HOAc. The fractions eluted with 50–60% EtOAc were methylated and analysed by GC-ECD (1% OV-17, 2 m × 2 mm i.d., N_2 flow rate 60 ml/min, oven 210°) and GC-SIM. For pea seedlings, the 50–60% EtOAc eluate was further chromatographed on 3 g of Celite 545 impregnated with 1.8 ml of 1 M NaPi buffer (pH 5.4) and eluted with a mixture of *n*-hexane and EtOAc. The fraction eluted with 60% EtOAc was methylated and analysed by GC-ECD and GC-SIM. Since 1′,4′-*cis*-diolABA and ABA methyl esters were not separated by GC columns of OV-1, OV-17 or SE-30, 1′,4′-*cis*-diolABA was detected with a 5% XE-60 column.

Assay of 1′,4′-t-diolABA in avocado fruits and pea seedlings. (+)-[$^2H_7$]-1′,4′-*t*-DiolABA was used as an internal standard. After a known amount of the internal standard was added to MeOH extracts, extraction and purification were done as described before. EtOAc-soluble neutral and acidic parts were used instead of the EtOAc-soluble acidic parts. When each sample was analysed, a standard curve was constructed to cover the 1′,4′-*t*-diolABA concn range expected in the samples (0–100 ng). The peak height ratio of *m/z* 244/*m/z* 250 was used in all calculations of the amount of 1′,4′,-*t*-diolABA.

MS and GC-SIM. GC/MS: Jeol DX-300 interfaced to GCG05. GC: 2% OV-1 (1 m × 3 mm i.d.), oven 210°, He 30 ml/min. MS: source 230°, jet separator 280°, 70 eV.

^2H-Incorporation was assessed by a full MS taken across the GC peak and calculated after correction for isotopic contributions. GC-SIM: 1% OV-17 (1 m × 3 mm i.d.), oven 215°, He 30 ml/min. MS: source 230°, jet separator 280°, 70 eV.

Metabolism of (+)-[$^2H_7$]-1′,4′-cis-*and* t-*diolABA and* (+)-[$^2H_6$] *ABA in pea seedlings.* Pea seeds were germinated and the seedlings were grown in a 10^{-4} strength Hyponex soln for 10–12 days. The seedlings (60 g) were derooted and placed in beakers. The labelled-compound soln at a concn of 10^{-3} M (10 ml) was added to the beakers and left to be taken up by the shoots for 2 hr. The shoots were grown in tap water for another 24 hr. The MeOH extracts of the shoots were concentrated and extracted with *n*-hexane. The aq. layer was acidified and extracted with EtOAc. The EtOAc-soluble parts were chromatographed on silica gel with a mixture of EtOAc and toluene containing 1% HOAc. ABA was eluted with 40% EtOAc, PA and 1′,4′-*t*-diolABA were eluted with 50% EtOAc, and 1′,4′-*cis*-diolABA was eluted with 60% EtOAc. Each fraction was analysed after methylation. Occasionally the PA was purified further by HPLC on ODS eluted with 40% MeOH containing 0.1% HOAc.

Metabolism of (+)-[$^2H_7$]-1′,4′-cis- *and* t-*diolABA and* (+)-[$^2H_6$] *ABA in avocado fruits.* Avocado fruits were used when they began to soften. They were cut in half and the seeds were removed. Longitudinal and transverse cuts (total, 170–180 g) 30–40 mm apart were made and on the newly exposed surface of each slice was applied 500 μl (Triton X-100: Me_2CO: H_2O, 1:1:8, by vol.) of 10^{-3} M ^2H-labelled compound soln. The treated slices were placed in a water-saturated atmosphere and covered with plastic bags for 24 hr. Extraction and purification were the same as for pea seedlings.

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