3-METHYL-5-(4′-OXO-2′,6′,6′-TRIMETHYLCYCLOHEX-2′-EN-1′-YL)-2,4 PENTADIENOIC ACID, A PUTATIVE PRECURSOR OF ABSCISIC ACID FROM *CERCOSPORA ROSICOLA*

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1. Introduction

Although the plant growth inhibitor abscisic acid (ABA) has been intensively studied by plant physiologists and biochemists, little is known about its pathway of biosynthesis. ABA appears to play an important role in plants subjected to water stress. Under water stress conditions ABA levels in leaves can rise by up to 20-fold and this increase in ABA may be important in regulating stomatal aperture [1]. Attempts to gain an insight into the biochemical mechanism whereby plants control their ABA levels have been sorely hampered by a lack of knowledge of its pathway of biosynthesis. Although two pathways, a direct C_{15} route and an indirect C_{40} route via carotenoids have been proposed, no reliable experimental evidence has been produced to substantiate either of these proposals. The stereochemistry of ABA biosynthesis in *Avocado* fruit is consistent with both suggested routes [2].

The major problem associated with the elucidation of the pathway of ABA biosynthesis in plants is the extremely low level of this compound found in plant tissues, even under conditions of water stress. Thus no intermediates on the ABA pathway have been identified to date. The observation that the plant pathogenic fungus *Cercospora rosicola* produces relatively large levels of ABA [3], has offered the possibility of determining the pathway of ABA in this organism. It may then be possible to check for the existence of a similar pathway in higher plants. This paper describes briefly the isolation of 3-methyl-5-(4′-oxo-2′,6′,6′-trimethylcyclohex-2′-en-1′-yl)-2,4 pentadienoic acid (1′-desoxy-ABA) from *C. rosicola*. We believe that this compound may be the immediate precursor of ABA.

2. Materials and methods

The fungus was sub-cultured on modified Miller's medium under fluorescent lighting at 26 ± 1°C. An aqueous solution (2 ml) containing ~25 μCi of [2-^{3}H]-MVA-lactone (spec. act. 745 mCi/mmol) was applied to the surface of the mycelium in each of 2 culture flasks 13 days after subculture. After 24 h the contents of the flasks were extraced in 0.2% acetic acid in ethyl acetate. The ethyl acetate was reduced to dryness, dissolved in 2% aqueous $NaHCO_3$ and an acid ether fraction prepared. This acid ether fraction was then analysed by HPLC as described.

High-pressure liquid chromatography (HPLC) systems used were:

(A) ODS-Hypersil (150 × 10 mm i.d.) eluted with a linear gradient of 20–100% methanol in 0.1 M acetic acid over 40 min at a flow rate of 5 ml/min.
(B) Hypersil (250 × 10 mm i.d.) eluted with chloroform/acetic acid (100/2) at 5 ml/min.
(C) Spherisorb (250 × 4.5 mm i.d.) eluted with hexane/iso-propanol (95/5) at 2 ml/min.
(D) Partisil (250 × 10 mm i.d.) eluted with chloroform for 10 min, then chloroform/acetic acid (100/0.5) at 5 ml/min.
(E) Partisil PAC (250 × 4.5 mm) eluted with hexane/ethanol (100/0.5) at 2 ml/min).

Gas–liquid chromatography (GLC) conditions used were: 3% OV-1 on Gas-Chrom Q (100/120 mesh); N_2 carrier-gas flow rate 40 ml/min, column temp. 220°C.

1′-Deoxy [^{3}H] ABA, 1 μCi (spec. act. 8.6 mCi/mmol) was applied to the mycelium in the same way as MVA. The ABA isolated from the medium was purified by HPLC on systems A and B and after methylation by HPLC on system C and finally preparative GLC.

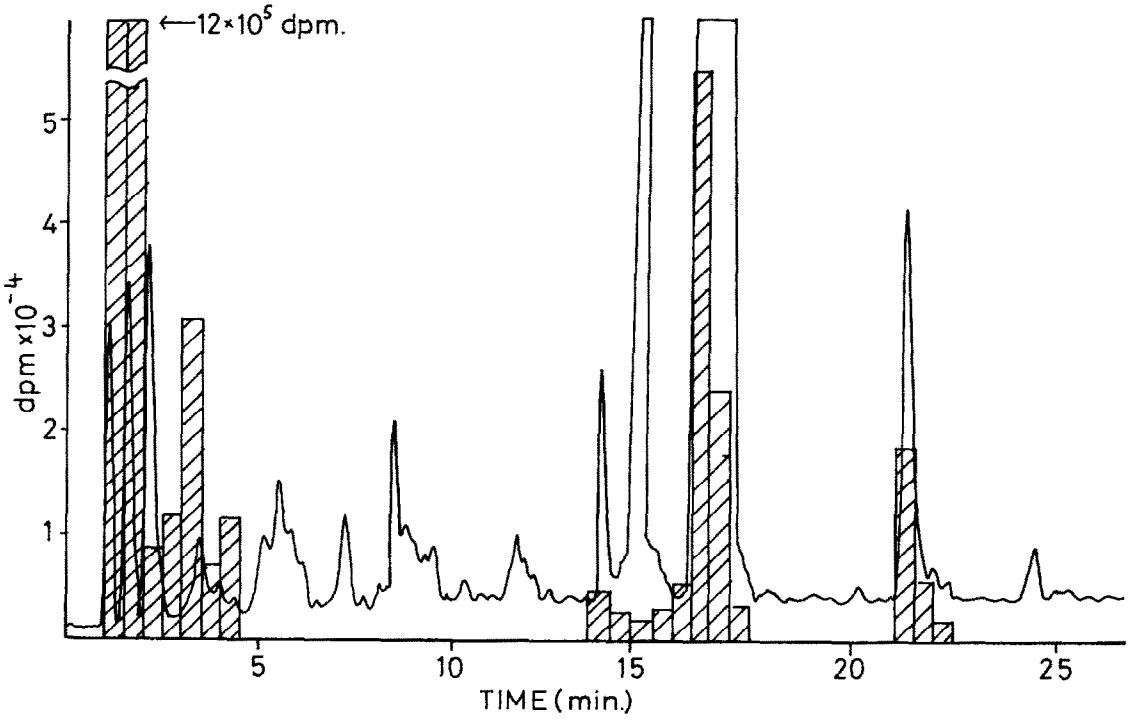
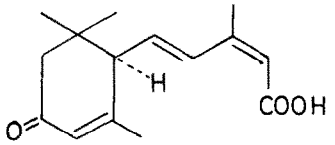


Fig.1. HPLC of acid ether fraction of [^{3}H]MVA feed using system A. Histogram represents radioactivity/2.5 ml fraction. Continuous line shows A_{265}.

3. Results

The distribution of radioactivity from MVA in the acid ether fraction after HPLC is shown in fig.1. The peak of radioactivity at R_f = 17.0 min co-chromatographed with ABA on HPLC on systems A and B, and with methyl ABA on system C and on GLC. The peak of radioactivity at R_f = 21.5 min was collected, methylated and examined by GCMS. Two major components were observed. The first of these was identified as methyl palmitate by its mass spectrum and retention time on GLC. The second component, eluting just after the methyl palmitate, exhibited major ions at *m/e* 174, 146, 125 (base peak), 119. The presence of these ions suggested a compound with an ABA-like structure. The quantity estimated by GLC was ~0.1% of the ABA.

The compound 40 μg was isolated and purified by HPLC on systems A and D. After methylation it was finally purified by HPLC on system E. The UV-absorbing material isolated by this large scale extraction co-chromatographed with the radioactive component of the initial [^{3}H]MVA feed. The mass spectrum of this



I

material exhibited a molecular ion at *m/e* 262 and major fragment ions at *m/e* 206, 174, 146, 125 (base peak), 119, 112, 105 and 91. The UV spectrum in MeOH had a λ_{max} at 267 nm with a shoulder at 240 nm. This data suggested the structure I. The PMR spectrum of the methylated material confirmed this structure. The *cis* configuration of the 2,3 double bond was indicated by the presence of a doublet at δ 7.72 (integrating for 1 proton) in the PMR spectrum. The ORD spectrum of I was virtually identical to that of (*S*) methyl-ABA. The proposed structure was confirmed by the synthesis of the methyl ester of I via the CrO_3 (pyridine)$_2$ oxidation of the 2-*cis* isomer of methyl-3-methyl-5-(2′,6′,6′-trimethyl-cyclohex-2′-en-1′-yl)-2,4 pentadienoate. The UV, MS and PMR spectra of the synthetic and isolated product were identical.

^{3}H-labelled I was synthesised from α-[^{3}H]ionone and was incorporated into ABA in 13% yield by the fungus. The [^{3}H]ABA isolated from the feeding experiment showed no change in specific activity on HPLC in systems A and B, as the methyl ester in system C and on preparative GLC.

4. Discussion

The presence of 3-methyl-5-(4′-oxo-2′,6′,6′-trimethylcyclohex-2′-en-1′-yl)-2,4 pentadienoic acid in *C. rosicola* which is actively synthesising ABA together with the incorporation of radioactivity from [^{3}H]MVA into this compound during the period when the [^{3}H]-MVA is being incorporated into ABA, suggest that the compound may be the immediate precursor of ABA. This suggestion is substantiated by the good incorporation of this compound into ABA by a culture of *C. rosicola* fed during the phase of rapid ABA production. Thus it is very likely that biosynthesis of ABA proceeds via successive oxidations of a 3-methyl-5-(2′,6′,6′-trimethylcyclohex-2′-en-1′-yl)-2.4-pentadienyl intermediate. This intermediate would arise via the desaturation and rearrangement of farnesol or a farnesyl derivative. This is in contrast to a route involving the carotenoid degradation product xanthoxin in which a 3-methyl-5-(2′,6′,6′-trimethylcyclohex-1′-en-1′-yl)-2,4-pentadienyl intermediate would most likely be involved. Further work is in progress to investigate the details of the proposed route.

References

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