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THE ORIGIN OF THE METHYL GROUPS OF ABSCISIC ACID

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Key Word Index—*Persea gratissima*; Lauraceae; avocado; abscisic acid; mevalonic acid; biosynthesis; isoprene methyl group.

Abstract—(+)-Abscisic acid (ABA), biosynthesised from (±)-[3′-^{14}C,3′-^{3}H]-mevalonolactone by avocado fruit, showed the same ^{14}C:^{3}H ratio as that of the precursor (3·0:3·0). After treatment with N NaOH, to exchange tritium from the methyl group attached to C-2′ of ABA, the ratio fell to 3:2. This is expected if the methyl groups at C-3, C-2′ and the pro-(*S*) position of C-6′ are each derived from the methyl at C-3 of one of the three mevalonate residues that comprise ABA. The ABA was then oxidised by a modified Kuhn–Roth procedure to give acetic acid which, after conversion into the *p*-bromophenacyl ester, showed a ^{14}C:^{3}H ratio (3:2·107) in accordance with the expected value.

INTRODUCTION

The carbon skeleton of abscisic acid (ABA) is composed of 3 isoprene units which are derived equally from labelled mevalonic acid when this precursor, in the form of mevalonolactone, is supplied to avocado fruit. The stereochemical positions that 7 of the carbon-borne hydrogen atoms of ABA occupy in the molecule of mevalonic acid have already been defined [1, 2]. Each isoprene unit should, according to previous investigations of the pathway of isoprene biosynthesis [3], carry a methyl group (Fig. 1) that was originally the methyl group at C-3′ of mevalonate, but this has not been demonstrated for ABA. The recent synthesis of (±)-[3′-^{14}C,3′-^{3}H]-mevalonolactone [4] made it possible to verify the postulate experimentally.

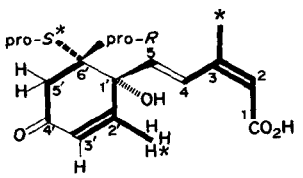
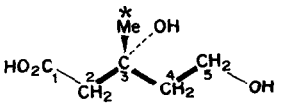


Fig. 1. (+)-ABA (Revised absolute configuration). The carbon atoms of each of the 3 isoprene residues that are believed to make up the carbon skeleton of ABA are linked by heavy lines. The 3 methyl groups believed to be derived from the methyl group at C-3 of mevalonic acid are starred and hydrogen atoms exchanged by *N* NaOH are drawn in.



(+)-Mevalonic acid. The 5 carbon atoms that become an isoprene residue are linked by heavy lines. The 3-methyl group is starred.

RESULTS AND DISCUSSION

A complete chemical degradation procedure for ABA has not been described but the hydrogen atoms can be dissected out with some specificity [2] by appropriate treatments. The hydrogen atoms of the methyl group attached to C-2′ (also those at C-3′ and C-5′) do not exchange in neutral or mildly acidic conditions [5] so that all the tritium in these methyl groups should be retained during isolation of the ABA, provided that mildly acidic conditions are maintained throughout.

However, these hydrogen atoms do exchange with the medium in N NaOH.

Labelled mevalonic acid supplied to avocado fruit is incorporated equally into the three isoprene residues of which the carbon skeleton of ABA is composed [2]. Consequently, if the methyl groups at C-3, C-2′ and the one at C-6′ of ABA are derived from the labelled methyl group at C-3 of [3′-^{14}C, 3′-^{3}H]mevalonic acid, then the ^{14}C:^{3}H ratio of ABA isolated under mildly acidic conditions should be 3·0:3·0 and this ratio would be expected to fall to 3·0:2·0 on treatment with alkali.

In fact ABA, biosynthesised from [3′-^{14}C,3′-^{3}H]mevalonate, and isolated entirely under acidic conditions, was found to have virtually the same ^{14}C:^{3}H ratio as had the original mevalonolactone (normalized to 3·0:3·0) when isolated in a radiochemically pure form [6]. In 2 experiments the normalized ^{14}C:^{3}H ratios of the ABA were 3:2·908 and 3:3·165. After treatment with alkali the ratios in 3 experiments fell to 3:1·998, 3:1·995 and 3:1·857. These ratios approximate closely to the value of 3·0:2·0 expected after the loss of tritium from the C-2′ methyl group but the results also indicate that two other positions in the molecule also carry equivalent amounts of label. The methyl group of ABA attached to C-6′ in the *pro*-6′(*R*) position has already been shown to be derived from C-2 of mevalonate.

It was possible to show that at least a considerable part of the tritium present in the exchanged ABA, and some ^{14}C from the position that had lost tritium by exchange was present in methyl groups by oxidizing the combined, exchanged sample of ABA by a modified Kuhn–Roth method [7, 8]. This procedure converts methyl groups, and the carbon atoms to which they are attached, into acetic acid with negligible loss of tritium [4]. The acetic acid can be conveniently isolated as *p*-bromophenacyl acetate.

The relatively small change in ^{14}C:^{3}H ratio between the sample of exchanged ABA (3:2·006) and the acetic acid isolated as *p*-bromophenacyl ester (3:2·107) is consistent with the label in ABA being present in methyl groups. If loss of tritium during oxidation is negligible then the methyl group attached to C-2′ (which carries no tritium) must have contributed to the acetate but uncertainties in the yield from the other two positions, which would be expected to carry both ^{14}C and ^{3}H, do not permit an unequivocable assignment of label to all three positions. The three methyl groups in ABA presumed to be derived from the labelled methyl group of mevalonic acid are in different environments and the difference between the observed ^{14}C:^{3}H ratio in the acetate (3:2·107) and the statistical value (3:1·8) is attributed to differing contributions from these three sites (Table 2).

Table 1. The ^{14}C:^{3}H ratios of ABA biosynthesised from (±)-[3′-^{14}C, 3′-^{3}H] mevalonolactone by avocado fruit in 3 experiments

	Observed dpm ^{14}C	Observed dpm ^{3}H	^{14}C:^{3}H ratio	Normalized ratio
(±)-Mevalonolactone	4200·8	43544·1	1:10·366	3:3
1st Experiment				
Methyl abscisate ($\frac{1}{4}$ of sample)	238·5	2396·7	1:10·049	3:2·908
Calculated value			1:10·366	3:3
Exchanged methyl abscisate	399·0	2749·5	1:6·894	3:1·998
Calculated value			1:6·911	3:2
Aqueous exchange medium for $\frac{3}{4}$ of sample (15·2 dpm ^{14}C and 104·8 dpm ^{3}H attributed to residual ABA have been subtracted)	—	2066·4	—	—
Calculated value for exchange medium	—	2257·8	—	—
2nd Experiment				
Methyl abscisate ($\frac{1}{4}$ of sample)	164·0	1793·7	1:10·937	3:3·165
Calculated value			1:10·366	3:3
Exchanged methyl abscisate	381·2	2562·8	1:6·723	3:1·995
Calculated value			1:6·911	3:2
3rd Experiment				
Exchanged methyl abscisate	181·5	1164·7	1:6·417	3:1·857
Calculated value			1:6·911	3:2

Table 2. ^{14}C:^{3}H ratios in acetate formed by the degradation of ABA

	Weight, (mg)	^{14}C dpm	^{3}H dpm	^{14}C:^{3}H ratio	Normalized ^{14}C:^{3}H ratio
Expected wt of acetic acid from ABA (2·2 mg) and added cold carrier (20 mg)	21·5				
Wt of acetic acid by titration of steam distillate	24				
Expected wt of *p*-bromophenacyl acetate	102·8				
Observed wt after chromatography	54·2				
dpm in abscisic acid oxidized		1990·0	13794·0	1:6·932	3:2·006
dpm in a 2 × recrystallized sample of *p*-bromophenacyl acetate (42 mg)		464·0	3377·9	1:7·280	3:2·107
Total calculated dpm in AcOH		1135·6	9267·2		

Base-exchanged samples of ABA from three experiments were combined, oxidized to acetic acid by a modified Kuhn–Roth procedure and the acetic acid formed was isolated as its *p*-bromophenacyl ester. All samples counted were spiked with ^{3}H and ^{14}C labelled toluene standards and corrected for quenching where necessary.

The results of these experiments, therefore, are consistent with the proposal that the methyl groups attached to C-3, C-2′ and the *pro*-(*S*) methyl group at C-6′ of ABA are derived from the methyl group at C-3 of mevalonic acid.

EXPERIMENTAL

The ABA was biosynthesised from (±)-[3′-^{14}C,3′-^{3}H]mevalonolactone (0·106 μCi ^{14}C; 1·065 μCi ^{3}H/μM) and isolated under acidic conditions throughout, as has been described before [2]. Mevalonolactone (1·11 mg) was fed to an avocado fruit (*ca* 160 g) and incubated for 24 hr in each expt. Methyl abscisate was radiochemically pure, as determined by chromatography of its $NaBH_4$ reduction products, and the hydrogen atoms of the 2′-methyl group were exchanged with those of the medium when the ester was dissolved in N NaOH (1 ml) or hydrolysed in 3N NaOH in EtOH–H_2O (2:1). Free acid was then rechromatographed on Si gel TLC plates in toluene–EtOAc–HOAc (50:30:4, eluted and counted in the scintillation soln used previously that gave 20% counting efficiency for ^{3}H and 49·5% for ^{14}C. Oxidation of the abscisic acid (2·2 mg) was carried out in aq chromic acid (2·5 g in 10 ml) by a modified Kuhn–Roth procedure [7,8], cold carrier HOAc (20 mg) was added to the reaction mixture before heating for 18 hr at 110°. The HOAc was steam distilled, titrated to pH with NaOH and evaporated to dryness. The NaOAc was dissolved in DMF (5 ml) with *p*-bromophenacyl bromide (100 mg) and stirred for 48 hr. The products were dissolved in 30 ml H_2O, extracted into Et_2O (2 × 20 ml) and chromatographed on 4 Si gel TLC plates in hexane–EtOAc (4:1) and the *p*-bromophenacyl acetate (54·2 mg) was recrystallised 2× from hexane to give the final sample (42 mg, mp 84–85·5°) which was counted.

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